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A MICROSCOPICAL STUDY OF CHANGES

DUE TO

Functional Activity in Nerve Cells.

BY

C. F. HODGE, Ph.D.

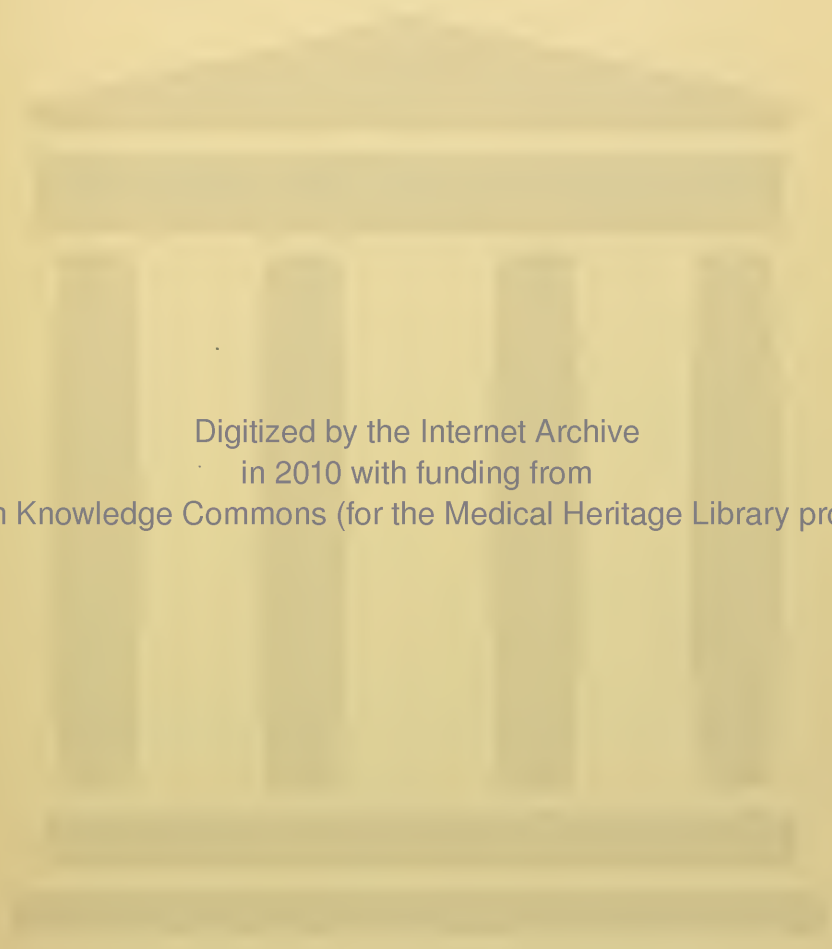
Reprinted from JOURNAL OF MORPHOLOGY, Vol. VII., No. 2.

COLUMBIA UNIVERSITY
DEPARTMENT OF PHYSIOLOGY
COLLEGE OF PHYSICIANS AND SURGEONS
437 WEST FIFTY NINTH STREET
NEW YORK

BOSTON :
GINN & COMPANY.
1892.



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TO
THE FOUNDER OF
THE MRS. JONAS G. CLARK FELLOWSHIP IN PSYCHOLOGY
IN CLARK UNIVERSITY
THIS MEMOIR IS RESPECTFULLY INSCRIBED
BY ITS FIRST HOLDER
THE AUTHOR

JOURNAL OF MORPHOLOGY.

A MICROSCOPICAL STUDY OF CHANGES DUE TO FUNCTIONAL ACTIVITY IN NERVE CELLS.

C. F. HODGE, PH.D.

Experiments upon a series of animals, including the frog, cat, dog, birds (pigeon, English sparrow, and swallow), and honey bee, with some observations upon chased foxes and pathological human material.

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I. INTRODUCTORY.

EXPERIMENTS for the purpose of studying changes in the cells of spinal ganglia upon electrical stimulation of nerves going to them, were begun in the biological laboratory of The Johns Hopkins University in the winter of 1887-88, and were there continued through the year 1888-89. During the two succeeding years the work was prosecuted in the neurological laboratory of Clark University. First of all, I wish to express my

gratitude, for his faithful supervision of the research during the whole time, to Dr. Henry H. Donaldson. Special thanks are further due to Clark University, which has provided me with the best obtainable apparatus and afforded generous opportunity for the prosecution of the work. It is with pleasure also that I acknowledge my indebtedness to Professor H. Newell Martin and to Professor Warren P. Lombard, for the privilege of using the apparatus of their respective laboratories.

The research has thus extended over a period of nearly four years. Results have been published from time to time in the *American Journal of Psychology* (23, 24, 25). Done from the standpoint of the physiologist and morphologist, rather than from that of the psychologist, I have not felt that it would be appropriate to give to its publication in a psychological journal the form best suited to the nature of the work. The reports so far have been thus necessarily incomplete. I desire, therefore, to give a full résumé of previous papers, thereby making the following a unified statement of the entire research up to date. This repetition is the more allowable, since, up to this point, the work is a logical unit. The logical sequence from the first has been determined, not by preconceived notions, but step by step by the outcome of the experiments. Thus, when I began by stimulating the sciatic nerve of the frog, I had little enough idea that it would bring me to a study of general nervous fatigue and restoration, and to the study of birds and honey bees at morning and at night. And results still warrant further prosecution of the work into the investigation of the more complex nervous systems of the higher animals and man in conditions of fatigue and disease.

II. THEORY AND PURPOSE.

To carry our knowledge a step farther into the working of the nerve cell is the sole object of the research. We already know that all the energy of the animal body comes directly from chemical changes which take place in the different tissues. The tissues have been specialized to perform certain chemical reactions, and in the individual cells we must find epitomized the function of the whole tissue. That is to say, did we but know all the processes which take place in a single nerve cell,

we should know or at least have the key to learn all of nerve physiology, from the action of the nervous mechanism (?) in an amoeba's protoplasm, through the entire animal series, to the activity of the human brain.

A certain fascination attaches to the study of the nerve cell, because it is associated with the higher activities of life. Sensation, intelligence, volition, are in some way dependent upon the integrity and healthful action of the cells of the brain, and many have been the theories, without foundation, concerning the working of the soul within its "material sanctuary." In fact, so many ideas, thoroughly unscientific in character, have appeared in this field, that it is with some slight danger that one undertakes to work in it even now. It is still a living sentiment that a man who meddles much with the brain is seeking the "seat of the soul," as De Cartes actually did, and as Charles Bell, nearly two hundred years later, was accused of doing. As a friend remarked to the author on beginning the work, "You will find no changes in nerve cells corresponding to those which take place in a gland. Changes are demonstrable in gland cells, because these produce a material secretion; you should find changes in muscle, because its action results in mechanical work; but the case of a nerve cell is different, its secretion is consciousness, a thing outside the equation of conservation of physical energy." As he put it, "The action of nerve cells is in a fourth dimension of space." However, transcendental objections to the contrary, the problem is simple enough. A nerve cell is certainly a minute speck of three dimensional, material protoplasm. Compared with the cells of other tissues it is often large, and compared with them, too, it is definitely characterized. A nerve cell is in general made up of a mass of granular protoplasm, enclosing a large nucleus, which exhibits a delicate reticulation and contains a prominent nucleolus. In a spinal ganglion cell, for example, all these characters are much more prominent than in any of the gland cells wherein functional changes have been observed. If a nerve going to a gland be stimulated, the cells become active, granules pass out of the cell protoplasm into the secretion, and the cell nuclei often undergo marked changes of appearance. If a nerve going to a ganglion be stimulated, if the cells have any function, why might it not be possible to demonstrate sim-

ilar changes in them? When changes in gland cells were demonstrated for artificial stimulation, the question arose, Why may not similar changes occur in the normal daily activity of the gland? So as changes due to artificial stimulation were noted in nerve cells, it was realized that if normal, similar changes should be found in the normal daily rest and activity of the animal.

While so much space in physiology is given to processes of digestion and nutrition, very little is given to those distinctively of rest. Indeed, in a leading physiology of the human body in this country, the subject of sleep is not treated, and even the word "sleep" does not occur, in coarse print, in the book. And yet what fact in physiology is more clearly indicated than that of the necessity of rest after activity? An animal is awake and active for, we will say, twelve hours. It then sleeps for twelve hours. The sleeping and the waking are dependent, without doubt in chief part, if not entirely, upon processes which are taking place in the cellular portions of the nervous system. To account for such profound functional changes, is it illogical to expect to find correspondingly great changes in structure? The necessity for rest in a gland cell is made apparent by its loss of substance. If nerve cells do not lose substance, or change in some way, why are we tired at night?

III. HISTORY OF RELATED WORK.

A knowledge of cellular activity which will enable us to apportion to each part of the cell—nucleus, reticulum, granulation, etc.—its peculiar role, to know the purpose for which it exists, and the work which it does for the common good, such knowledge can only be gained by study of physiological activity in cells, and not only in reproductive cells, but in cells of all kinds and functions. It has long been my purpose to sift all the work that has ever been done upon the line of changes in cells due to functional activity, and to glean out whatever consensus of opinion may have been reached. Pressure of other work, and, while in Madison, the lack of all literature, either current or classical, has made it impossible to carry out this plan as fully as desired. A few points require discussion, however, before passing to a consideration of my own experiments.

There is, indeed, little enough consensus. In no field of biology is there such a Babel of discord, and the reason for this is obvious. The material of observation here is not permanent form, but flitting, vanishing, ever-changing phases of action. What one observer sees is gone before another observer can confirm it. Further than this, and aside from the difficulty of making exact observations, the causes which modify or influence cellular activity are little understood. Hence causes which might account for difference in results are likely to be overlooked, and results themselves are claimed to be different; considerations like the above will be of assistance as we proceed.

What Minot (52, p. 98) would say of the whole organism is true of its individual cells. No process is more characteristic of living protoplasm than growth. And growth in a metazoan may be due to either cell-multiplication or to cell-growth. The first is plainly reproduction. May not also cell-growth (57, p. 23; 51, p. 439) be considered in essential nature, a process of the same kind, in which the increment of matter gained is used for some other purpose by the cell than that of reproducing another cell like itself? An amœba, or a tissue-cell, grows and then divides into two cells, we will say, of exactly the same kind, and half the size of the original. A working tissue-cell grows to twice normal size, but, instead of dividing, it now throws off half of its substance, let us say, in the form of zymogen granules. That is to say, the cell has become specialized, so that instead of dividing into two equivalent parts it divides into two unequal parts; the one remaining as the original cell, the other passing off to do the work for which the cell has become specialized to perform. If there is any truth in this view, we should expect to find the same mechanisms which mediate cell-reproduction active in cell-function. Exactly what the reproductive mechanisms of a cell are, despite the vast amount of work devoted to the subject, is still a matter of controversy. I shall attempt no special discussion, and hence shall refer to but two or three papers which bring out points of immediate use to us, bearing upon the general subject of cellular activity.

In the process of division no part of the cell is likely to show such active changes as the nucleus. In fact, the nucleus is not infrequently called the reproductive organ of the cell. May not

the nucleus be equally active in the growth of protoplasm for use in functional activity, as well as for cell-division?

An experiment of Boveri (7) in confirmation of observations by the Hertwigs (22) throws some light upon functions of the nucleus. The experiment consists in fertilizing a denucleated fragment of a sea-urchin's egg with a spermatozoan of another species; Rauber's (70) experiment, upon toads' and frogs' eggs, repeated upon a form where success was possible. Rauber wished to ascertain the relative influence of nucleus and protoplasm in the determination of species. Boveri carried the experiment far enough to demonstrate that such denucleated fragments developed into pure male-type embryos. That is, the female protoplasm had no influence. It served simply as food matrix in which the male nucleus could develop. If a female nucleus is present in the fertilized fragment, a hybrid is developed. So that Boveri is confident in concluding that the nucleus alone carries specific characters from parent to offspring. Watase (83, p. 262), who gives great prominence to the part taken in cell-division by a portion of the protoplasm (archoplasmic spheres and filaments), coincides with the above opinion in the following words: "It is now quite generally conceded that the nucleus of the fertilized ovum contains all the hereditary characteristics of the parent organisms."

If, then, a single microscopical nucleus is capable of determining the form, nuclei, and protoplasm of all the cells of an animal, *a fortiori*, the nucleus should certainly determine the protoplasm of its own cell. The truth of this is seen in the development of any tissue (53, p. 17). At first there is a mass of nuclei with scarcely a trace of protoplasm; then around each nucleus protoplasm is gradually laid, until, in form, amount, and structure, the adult cell is attained. Whence comes this protoplasm, if it is not developed from the nuclei? What are nuclei doing in solid heaps unless busy making protoplasm? From the role which they play in a developing ovum, it is plain that nuclei are things too vital and active to be lying around idle.

This brings us to a principle which should underlie all study of cellular activity. It may be stated in two ways.

(1) *In any specialized tissue, seek for changes due to functional activity in the structures most prominent in the cells of that tissue.*

(2) *Conversely, a cell becomes specialized to perform a certain function only by an increased growth of certain of its parts.*

Thus reproductive tissue is most richly nucleated. Possibly even more richly nucleated is cellular nerve tissue. In gland tissue, nuclei are quite prominent; but the characteristic of a gland cell is its granulation. From a red blood corpuscle the nucleus may be entirely lacking; the reticulum, stroma, is scarcely discernible; all that is left is a highly specialized protoplasm; and here the only change we know is that from reduced to oxy-hæmoglobin, and *vice versa*. Intermediate between the last two stand such mechanical tissues as cartilage, bone, connective tissues, and, we must add, nerve fibres and muscle. These, when adult, consist chiefly either of comparatively inert intercellular substance, or of what we may consider a special development of a fibrillar cell-reticulum. And, aside from mere changes in form, muscular contraction, reticular contraction as seen in amœba and ciliated epithelium, etc., and possibly the changes in archoplastic spheres and filaments, where do we find changes in the reticulum of cells?

In running through the list of tissues, therefore, to ascertain what changes connected with functional activity have been observed in each, we shall watch for the following points: —

1. Changes in nucleus.
2. Changes in protoplasm } *a.* Granulation.
 } *b.* Reticulation.

I question the advisability of further refinement at present.¹

¹ It is immaterial to my present purpose, although it may not be to a future one, whether we consider the structure of protoplasm to conform to any of the views advanced since the "structureless slime" of Dujardin and Von Mohl. Protoplasm must be something more than this. Lymph is constantly soaking through it, and plus this are certainly granules of some sort and a fibrillar meshwork of some kind. In this research I am not using sufficiently high powers, or sufficiently special methods, to make it a matter of importance whether protoplasm is the zoogloea of special bacteria of Altmann, or the foam of Bütschli. I therefore adhere to the old familiar view of Brücke, Arnold, and Max Schultze.

With regard to the nucleus, the writer has often wished that he had applied methods which would have enabled him to follow the substance, *chromatin*, a little more closely. This might have been possible as it was, had there not been so many different kinds of safranin in the market, with the exception of one sample, none of which stained chromatin properly. This may be remedied in future, and the matter does not concern us vitally at present

Reproductive Tissues.

No description of the formation of spermatozoa is necessary. Views as to details differ among different authorities (39, p. 900; 67, p. 688; 22, p. 17); but all are agreed as regards nearly everything that touches our point of view. In general, the nucleus is transformed into the head and the protoplasmic reticulum develops into a vibratile flagellum. The head assumes peculiar forms in different types, but whether it shows any increase or decrease in size I am unable to say. Processes of division have recently been observed, in which a portion of the head is extruded after the fashion of polar bodies (5).

The ovum also presents features of interest. Here both nucleus and protoplasm increase in size, often at the expense of surrounding cells (18, pp. 5 ff.), the nucleus in maturation suffering a reduction to one-fourth its chromatin by extrusion of polar bodies (63). Besides changes in nucleus and food material the reticulum often assumes a peculiar structure, to form the "zona radiata" around the outside of the ovum.

It may be well to bear in mind all phases of protoplasmic and nuclear activity passing by the names of oökinesis, cytokinesis, or karyokinesis. Let us see if anything in the functional activity of other tissues may be found to resemble these processes, or those of direct nuclear division.

Gland.

Of importance in their influence upon theories of secretion were the first experiments of Heidenhain. Secretion could no longer be thought of as the "straining off" of Malpighi (47, I, p. 464), or the "diffusion stream" of Deutroschet, when the activity of the cells themselves had once been demonstrated. Heidenhain (19, 20, 21) found that, as the cells secreted, their appearance changed in a marked degree. In general, a granular zone next the lumen disappeared, leaving the cells shrunken. The nucleus in the meantime, from being small and irregular in outline, became swollen. Protoplasm grew again, and from its substance arose a new zone of granules. Sooner or later the secreting cells go to the ground and new cells spring up to take their places.

The evidence for this last point is, however, questioned by Langley, who strongly advocates the opposite view, that after secretion, and in fact during secretion, the same cells refill with protoplasm and zymogen granules, and so on indefinitely. The facts thought by Heidenhain to indicate cell-renewal are given another interpretation by Langley (34, p. 676). He also questions whether the nucleus actually swells, and maintains that it simply appears larger in proportion to the greatly shrunken cells. In the same paper (p. 698) Langley emphasizes another point of great importance to us, viz. that processes of rest and activity, anabolism and katabolism, go on in the same cells at the same time. Hence the appearance of a cell at any time depends upon whether one or the other process is in ascendancy. This may account for the fact that one observer (62) has found that the cells of the gastric glands in the frog *increase* in size for twelve to eighteen hours after feeding, and then gradually resume their normal size. This observation, however, stands alone, and during his twelve years of close study Langley has seen nothing to confirm it. In general, Langley makes little of changes of the nucleus.

Seiller (76), in a special study of mucous cells, by most recent methods, supports the view of Langley, that goblet cells do not perish in secretion, but regenerate their protoplasm.

Great prominence is given to the action of the nucleus by Ogata (60) in his work upon pancreas secretion. A body with peculiar staining properties, plasmasoma, arises in the nucleus, and migrating out into the protoplasm may give rise to a mass of zymogen granules; or it may develop into a new nucleus, form a new cell about it, and then produce zymogen granules. That is, the process is chiefly reproductive in character. There may be a stable mechanism in the cells which can manufacture zymogen granules, but under special stimulation this is not sufficient (p. 430). Here, moreover, the reproductive process is different from ordinary cell-division, in which both cells live; the old cells in this case dying away. It is different also from the fact that there is nothing comparable with karyokinesis; nor does the formation and migration of plasmasoma resemble direct nuclear division. Kühne and Lea (33) saw granules in living cells of triton's pancreas streaming from the neighborhood of the nucleus toward the lumen (21, p. 203);

and, in addition to this, Ogata (60, p. 432) observed them in the act of passing out of the nucleus. Platner (64) does not confirm Ogata's observations; finding instead frequent cases of nuclear budding (*Kernsprossung*) after good feeding. His method, however, being so different from that of Ogata, renders minute comparison of results impossible.

An additional point of interest in pancreas secretion is made by Oppel (61); viz. that the nucleus from being clear and reticular in the resting condition shrinks and comes to take a dense homogeneous stain after secretion. Other changes in the cells are like those already described.

Van Gehüchten (14, 15), on the other hand, is strongly opposed to the view that the nucleus suffers any change during secretion. In case of the digestive cells of *Ptychoptera* larva, which he studied, it is in fact often thrown out with the secretion. In this case the cell dies, so that he concludes that the nucleus is essential to cell life, but not to secretion. How the cells are renewed is not observed; but nothing like cell-division is present. It is a little strange that, while Van Gehüchten argues against any change in the nucleus, he figures, side by side, in apparently similar cells, nuclei of most diverse sizes, some nuclei being easily twice the diameter of others.

Something similar takes place in mammary glands; but here the nuclei actively divide, and a part passes out into the secretion. No exact measurements exist, to my knowledge, but reference to the figures usually given (67, p. 723; 39, p. 391; 21, p. 383) reveals the fact that the nuclei are out of all proportion larger in the active than in the resting gland. Here, then, would seem to be found a correlation between size of nucleus and secreting activity of cell. If these nuclei did not increase in size, Van Gehüchten's statement, that nuclei have nothing to do with secretion, might have a more general application. The secreting cells in case of mammary glands show also great variations in amount and constitution of protoplasmic contents during the different phases of rest and activity.

For the cells of the liver both Heidenhain (21, p. 222) and Langley (35) describe a marked set of changes in the protoplasm, similar in the main to the changes in other glands. Heidenhain (21, p. 224) says that the nucleus is variable in appearance, but does not go into detail.

Secular changes in liver cells of frogs have been studied with great care by Alice Leonard (41). The cells are found to vary greatly in size at different seasons, reaching a maximum in November, and shrinking to less than one-fifth this size by April. The nucleus, on the contrary, is smallest in November, $6\ \mu$ in diameter, and largest, $7.6\ \mu$, in April. The protoplasm may be said almost to disappear during the winter, pigment in the cells showing an increase in amount at the same time. By this extreme change, an action of different stains upon constituents of the cell-protoplasm is brought to light; viz. that eosin (41, p. 34) stains carbohydrate, and nigrosin albuminous material.

There is seen to be little agreement as to the action of gland cells, and more can scarcely be expected as to results until methods become better known, more precise, and more consensus as to their use is reached. So far little more can be said to be established than that during rest the cells become filled with granules, and that during secretion these granules pass out, generally leaving the cell shrunken. A few observations indicate a probability that these granules arise in the nucleus. One writer (60) affirms the fact. The fact that nuclei are sometimes extruded during active secretion, as occurs in mammary glands and those of the digestive tract in insects (Van Gehuchten), is not necessarily opposed to this view. Whether the nucleus swells, or shrinks, or changes in staining properties is a question of dispute.¹

Muscle.

"A simplified view of the histology of the striped muscle fibre" advanced by Melland (49) in 1885 is the one adopted in the following discussion. According to this we have to deal with a highly specialized cell-reticulum with fibrils arranged in cross and longitudinal series. This is supposedly the contractile mechanism. Between the meshes of this reticulum is a structureless, semifluid muscle plasma. Scattered through the muscle substance or lying just underneath the sarcolemma are

¹ From the first the writer has intended to repeat the more important experiments in this field of gland histology, and until an opportunity for doing so presents itself further discussion of the subject will hardly be profitable.

inconspicuous (for adult muscle) nuclei, embedded in a little granular protoplasm.

Before proceeding it may be well to ask in which one of the above elements we should expect to find changes due to metabolic processes. From the axioms with which we started out, the reticulum being the characteristic feature of the tissue, if any change occurs it should occur here. We should certainly not expect to find any change in the nuclei. For, aside from their insignificant size, the nucleus has never been found to take any part in the function of contractility.

Any change, then, must be sought in fibrillar or interfibrillar substance. From the physiological fact, that little or no increased nitrogenous waste occurs from increased muscular work, we reason to a comparatively stable contractile mechanism in muscle, comparable to the iron-work of a steam-engine. We could hardly expect to find any change in a mechanism of this sort from a single day's work. We are therefore confined to the interfibrillar plasma, and here we undoubtedly have active metabolic changes; but the lack of definite granulation must add greatly to the difficulty of demonstrating visually any processes which may take place.

As might be expected, muscle tissue has been worked along this line with little success.

Du Bois-Reymond (11, pp. 11-72) discovered, as he at first supposed, marked changes due to fatigue. These consisted in the breaking up of the muscle substance into irregular lumps; or, with entire loss of fibrillar structure, into fine granules. On further experiment, however, he found that the phenomenon could be produced by simple stretching of the muscle. It occurred in equal amount whether the muscle was stretched, or stretched and stimulated. So that he concludes by saying (11, p. 72) "that frogs' muscles which were stimulated to complete exhaustion, as far as the appearance of their primitive fibres goes, are the same as muscles which have not been stimulated."

Again, Roth (72), in a most heroic series of experiments in which muscles of frogs and rabbits were stimulated *in situ* continuously for five, ten, and even twenty days, succeeded in demonstrating chiefly such changes as occur in pathological degeneration of muscle. The muscle substance became vacuo-

lated in some cases, in others not ; was broken up into lumps and granules which had lost fibrillar structure in part or altogether, and showed waxy degeneration. Some fibres exhibited the discoidal breaking up of muscle substance commonly seen in typhoid fever. That is, the mechanism was broken, not exhausted. The muscle nuclei showed no change whatever. Possibly the vacuolation which appears in some instances may be reckoned as genuine fatigue effect.

Under this head I may call attention to a few points in the metabolism of another mesoblastic tissue ; viz. the blood.

Alice Leonard (41, p. 39) points out the fact that the blood is greater in amount in November than at any other season, and the red corpuscles stain bright red with eosin at this time. During the winter they take the stain less and less, become smaller, until the minimum is reached in May. By July they begin to enlarge again and to take the stain. The nuclei, moreover, stain differently at different seasons and are found to differ both in structure and form, staining in some corpuscles densely, in others showing the usual reticulum. They may also appear shrunken and irregular or oval and clear.

Something similar for mammalian corpuscles while still nucleated is pointed out by Howell (26). Immature erythroblasts are nucleated red corpuscles having a large reticulate nucleus and a small amount of hæmoglobin. These divide by karyokinesis for several generations until finally a form is reached, the mature erythroblast, having a smaller densely stained nucleus and large amount of hæmoglobin. When the nucleus has lost its reticulum, no further division is possible, and it is then extruded from the corpuscle to be dissolved in the plasma.

Nerve Tissue.

All nerve cells are phylogenetically cells of the epiblast. In any section of skin, from the deepest layer of columnar cells to the horny scales at the surface, we may observe a series of changes which have a deep physiological significance. Here we have at a glance the life history of an epithelial cell. Is it the story of a cell being born, growing to maturity, and dying of old age ? It may be so. Or is it the case of a cell being crowded away from its supply of nourishment and dying of starvation ?

This also may be true. Is it the case of a cell doing its work, and, under the hail of changes which the external world showers upon it, dying of fatigue? And this may be true. So that we have epitomized in a single row of cells three great problems of life: its period of duration, struggle for existence, and fatigue.

No series of changes anywhere in the body have a more direct bearing upon changes during the life history of a nerve cell than this series in a cell of the epidermis. The cells begin life with a large nucleus and little protoplasm exactly as a nerve cell does. Protoplasm grows much, nucleus grows somewhat, like a developing ganglion cell. Farther on, the nucleus begins to shrink, looses its reticular structure, and disappears when the change of the cell from protoplasm to horn has been completed. A similar set of changes have been described for the atrophy of nerve cells, the end product of course being different in the two cases. And whether the life history of nerve and epithelial cells is comparable to the end remains to be seen when the changes due to aging have been fully worked out for the nerve cell. We clearly have in the epidermis functional activity involving the destruction of cells. This fact finds a natural explanation in the superficial position of the cells. Why this should not be the case with nerve cells will be discussed later.

A single case of marked changes in epidermal cells due to artificial stimulation is given us by Kodis (28) for the tadpole. Kodis finds that one hour's electrical stimulation of the skin occasions a shrinkage in the epidermal nuclei of nearly sixty per cent (figuring volume of nuclei from measurements taken from Kodis' drawings) (compare Taf. III, Fig. 34 with Taf. I, Fig. 1). The nucleus at the same time becomes granular and dark. The nucleolus also shrinks and ceases to stain bright red with safranin as it does in the resting cells. With the exception of this last, which is not so clearly demonstrated in my specimens, the changes are quite similar to those taking place upon stimulating the nerve of a spinal ganglion.

We shall enter now the vast field of nerve literature with an eye single to the subject in hand; viz. microscopical changes connected with functional activity. Only so much of morphological interest will be cited as is necessary to supply a physical basis for physiological action. In brief, the conception of the minute structure of nerve elements which has satisfied every

condition of my research is that furnished us by Max Schultze (75) and confirmed later by the work of Kupffer (31), Boveri (6), and Joseph (27). My preparations do not in any way support the tubular theory which Nansen (56) has drawn from his observations upon the nerves of invertebrates. This conception is that the axis cylinder consists of a bundle of fine fibrils floating in a plasma. All fibrils arise as outgrowths of nerve cells (29, p. 51), and are seen continued into the cell as the fibrillar reticulum of the cell-protoplasm. In the cell, and to some extent in the nerve fibre, granules occur between the fibrils. Those in the fibre are exceedingly fine, in the cell are generally coarse and so densely packed as to hide the reticulum altogether. We have thus at least the two things necessary for a nerve mechanism: the fibril to conduct, and, in close touch with this, a granular substance, changes in which may serve to originate or modify the nerve impulse. In addition we have a nucleus and nucleolus, proportionally as large or larger than the nucleus of a growing ovum. Thus all the elements of cell structure, nucleus, granulation, reticulum, are highly developed in the ganglion cell. May we not then expect to find changes like those of an ovum in the nucleus, and changes in the granular contents like those occurring in gland cells? We have no ground to expect to find any change in the fibrillæ themselves.

A good deal of work has been misdirected to the study of so-called pathological changes in nerve cells. I say misdirected, because until normal processes are known it is clearly impossible to draw the line between what is normal and what is abnormal.

A careful statement of pathological changes is given by Obersteiner (59, pp. 112-116 and 125-129). The gross process of degeneration in a nerve fibre is comparatively simple and too well known to require description. For the ganglion cell pathological changes are exceedingly varied. Of the nine varieties described by Obersteiner I shall at present file a word of caution against two. Simple atrophy, he says, begins by shrinkage with loss of structure of the nucleus, its outline becoming jagged, followed by shrinkage of cell, disorganization of processes, and finally ending, it may be, in the disappearance of the entire neuron. Again, vacuolation is given as a pathological change in cases of inflammation. Although Obersteiner is careful to

state that the amount of vacuolation must be considerable and its presence in the cells general, if we are to consider it a sign of pathological change, still I doubt if pathologists realize the amount of shrinkage and vacuolation which may occur in normal fatigue. As to the other forms, fatty or pigmentary degeneration of chronic atrophy in paralytics and drunkards, sclerosis, the calcification of *plaques jaunes*, etc., fragmentation of nuclei, etc., there is no doubt as to their pathological nature.

For the spinal ganglia Angelucci (4) in cases of chronic and acute myelitis and paralytic insanity describes among other changes a shrinking up of the nucleus, its outline becoming "Stelliforme," and finally it disappears. Similar appearances are found by Müller (55, Pl. I, Fig. 7) in normal ganglia and described without explanation as degenerated nuclei. Rosenbach (68) obtained about the same results, shrinkage and disappearance of nucleus with vacuolation of protoplasm, from the spinal ganglia of dogs which had been starved. And Lewen (42) finds the same appearances in the ganglion of the vagus nerve in consumption and exhausting disease of heart and stomach. He attributes them to deficient nutrition. R. Schulz (74) from examination of twenty cases draws the generalization that pigment increases in the ganglion cells of the spinal cord with age and impaired nutrition. Whitwell (84) describes vacuolation in the nucleus of both large and small pyramidal cells of the cortex in cases of dementia, especially when following epilepsy. Mamurowski (50) describes a case of death from progressive paralysis due to alcoholism, in which the peripheral nerves showed degeneration, but no change was observable in either brain or spinal cord. The above represents but a few of the observed changes which might be sifted out of the literature of the subject.

In the line of experimental pathology, beside the experiments just referred to, Rosenbach (69) has found degeneration of fibres and atrophy of ganglion cells in the cord of dogs in the neighborhood of compression. In fact, the Russians have done considerable work of this kind, and from the title of several of their papers, I had expected to find the subject of normal fatigue treated. I was, however, fortunate enough to obtain a reading of the more important articles, and found them, in purpose and idea, pathological. For example, Anfimow (1)

studied the changes in the central nervous system of animals dying from varnishing the skin. A constant symptom, he notes, is hyperemia of spinal cord and brain with numerous capillary hemorrhages in the gray matter, especially of cord and medulla. Extreme vacuolation is the most characteristic change in the cells.

An exhaustive research of Sadovski (73), under title, "On the changes of nerve centres due to peripheral irritation," has for its purpose "to ascertain whether pathological changes in the centres can be induced by irritation of a nerve." He accordingly stimulates, or better, irritates a nerve, generally by ligature ("from 10 to 71 days"), and thereby succeeds in causing neuritis with formation of a "knot about the size of a pea" and peripheral, not any central, degeneration of the nerve. Microscopical examination of the ganglia, using those of the uninjured side for controls, showed for most of the cells no difference. Many cells, however, on the operated side exhibited great vacuolation and shrinking of protoplasm from the capsule. The nuclei of the altered cells he describes as "oval instead of round, densely stained, sometimes shrunken so as to leave a space between nucleus and protoplasm, and zigzag in outline." In later stages no trace of nucleus is present. In some of his experiments Sadovski employs electrical stimulation, and it is difficult to understand from the ground of my own experiments how, in Group III, experiment 5, for example, a moderate stimulation of only fifteen minutes daily for twenty-one days could have produced the vacuolated protoplasm and shrunken and atrophied nuclei that he describes for it (73, p. 30). The nerve, auricularis magnus, in the ear of a dog was stimulated through the skin. In explanation, Sadovski advances the view that any such additional irritation causes the nerve cells to break down more rapidly than they are able to recover, and a gradual "atrophy" takes place. Hence he affirms "it is possible to demonstrate morphological changes in nerve cells due to excessive activity." He says nothing of *normal* activity.

Somewhat similar to the last is the research of Mrs. Ternowski (80) upon "Changes in the spinal cord occasioned by stretching the sciatic nerve." Among other things, such as hyperemia, etc., vacuolation and atrophy of ganglion cells is noted in both anterior and posterior horns. This is opposed

to Vulpian's (82) experiments upon guinea pigs, in which he was unable to discover any changes in the spinal cord.

Upon the purely physiological histology of nerve tissue little work has been done.

For the nerve fibre, Kühne notes a change in the axis cylinder, a disarrangement and shrinking together of the fibrilæ with the appearance of vacuoles between them, in the nerves of the nictitating membrane of the frog due to only ten minutes' unipolar stimulation of the nerve root within the skull (30, p. 56; Taf. D, Fig. 64). But physiological evidence has been piled up by Bernstein and Widenskii, and later by Bowditch (8 and 9) and Szana (79), all to the effect that a nerve fibre is not susceptible of fatigue. That an excised nerve dies more quickly when stimulated than when left at rest, is conclusively proved by Lee (40); and why this should occur, if no change associated with activity takes place, is difficult to explain.

For the nerve cell, possibly the observations of Svierczewski (78), as long ago as 1869, have a physiological bearing. This observer studied the cells of the frog's sympathetic ganglia kept alive in aqueous humor or lymph, and subjecting them to different conditions, observed the effects. From what more recent work is bringing to light, it is significant to note that active changes were discovered only within the nucleus. The nucleoli were observed to wander about in the nucleus, sometimes in a most lively fashion, for as long as twenty-four hours. On exposing the cells to carbon dioxide, a finely granular precipitate suddenly formed within the nucleus, which redissolved on treatment with oxygen or hydrogen ("paraglobulin-reaction"). This process was accompanied, under certain conditions, by a marked shrinkage, the rounded form of the nucleus being altered to an irregular or "zickzack" outline, the nucleolus at the same time being lost to view.

Somewhat similar observations were made by Freud (13) upon the living ganglion cells of *Astacus*. He describes shreds and angular-shaped particles which change form and position within the nucleus.

The only paper devoted to the exact problem in hand was written in 1889 by Bohdan Korybut-Daszkiewicz (32). The author states the exact question: "Is the activity of the central nervous system accompanied by changes recognizable with

the microscope?" He proceeds to answer the question under the idea that staining reveals much finer differences than changes of form. This determines his method, which consists in choosing two frogs of the same weight and sex, the one to be experimented with, the other to use as control. He then stimulates by induction, shocks the eighth nerve of one frog for one hour, keeping the control frog as quiet as possible during the same time. The spinal cords of both are now removed and hardened in corrosive sublimate solution and alcohol, and sections are made through both, opposite the origin of the eighth nerve. The sections are stained on the slide with hæmatoxylin, nigrosin, eosin, and safranin (the Gaule combination), in the order named. In *some* cases, the author states, sections of the two cords were treated on the *same* slide. Here, again, interest is attracted to the nuclei. By a difference in staining these fall into two categories, the red and the blue, and a greater proportion of the nuclei stain red in the cord of the stimulated frog. A count of all red and all blue nuclei, in a large number of sections, shows that from 3.31 to 3.66 times more nuclei stain red in the stimulated than in the unstimulated frog. The results are derived from but four frogs, two stimulated and two control.¹

Reproductive tissues, gland, muscle, and nerve have thus been worked, with a purpose of demonstrating microscopical changes connected with functional activity. The above is itself a résumé. I shall not attempt a résumé of a résumé. I wish, however, to gather in a few words the ideas having special bearing upon my own work.

1. In connection with reproduction, the nucleus of the fertilized ovum determines the form of the whole animal. Each nucleus determines the protoplasm of its own cell.

2. Protoplasm may be of the nature of a stable mechanism,

¹ For reasons detailed in a previous paper I do not place entire confidence in the above results (24, p. 384). Such a difference may be due to the frogs for so small a number of cases, but is more probably due to a difference in thickness of sections, as I have found that thick and thin sections stain differently in exactly this respect, the nuclei near the surface of the section staining red, those deeper down staining blue. Hence the thinner the section the greater the proportion of red-stained nuclei, and in equal areas of section Daszkiewicz finds nearly 400 (4127 to 3759) less nuclei in the stimulated cord. This would indicate that these sections are thinner, and here he finds the preponderance of red nuclei.

formed by the nucleus, under normal circumstances, once for all (muscle, connective tissue, etc.), or it may be unstable and be formed continuously as used up (gland). In the former case, nuclei take a subordinate place in the tissue after the mechanism is built. In the latter case, the nuclei retain a prominent position throughout the life of the tissue. Granules have been observed (Ogata) streaming out of the nucleus into the cell-protoplasm, and while the many may not have applied methods suitable for demonstrating this, nothing *has been seen* which renders the fact improbable. For an instructive discussion of this most vital of all points, I cannot do better than refer the reader to De Vries' (10, pp. 180-187) intracellulare Pangenesis. He will there find discussed the views of Haeckel and the Hertwigs, Flemming and Strasburger, Tangle, Haberlandt, Korschelt, Pringsheim, Schmitz, Nussbaum, Gruber, Hanstein, Weisman, Klebs, and others, all of whom bring here a point and there a point to prove that out of the nucleus comes everything of structural significance in the protoplasm. Read also Altmann, *Elementarorganismen* (2), and *Die Structur des Zellkerns* (3).

3. In no cells are nuclei more prominent than in ganglion cells. Changes have been observed (Svirczewski and Freud) within the nuclei of nerve cells, and, possibly, differences in staining. Granulation also forms a characteristic feature of ganglion cells. This resembles in appearance the unstable mechanism of gland cells. If this outward resemblance is real, we shall find changes in granulation also.

IV. EFFECTS OF ELECTRICAL STIMULATION.

Method.

Throughout this series of experiments, the spinal root ganglia were used. The scheme of procedure was to stimulate a nerve going to one or more of these ganglia on one side of the animal, leaving the corresponding ganglia of the other side at rest, to use as control. To avoid confusion, the right side was used for stimulation, the left for control. The stimulated nerve was never divided, so that the contraction of its muscles could be used to indicate the healthy condition of the nerve. If a nerve

is conducting impulses peripherally to its muscles, it may be taken for granted that it is conducting impulses in like manner centrally to its ganglion.

In general, as a means of stimulation, the ordinary combination was used of Du Bois Reymond coil, platinum electrodes, and bichromate or copper sulphate cell; and the strength of stimulus was determined within physiological limits by touching the electrodes to the tongue before beginning to stimulate. For the first few experiments the animal was put under the influence of curare and the stimulation was continuous. Failing of any results, the use of curare was abandoned (39, p. 523) and intervals of rest were allowed. At first this was managed by placing a key in the circuit and making and breaking the circuit once a minute by hand. In later experiments, this was relegated to clockwork, which spaced the intervals with more precision and removed the chief feature of irksomeness from the operation.

At the end of the desired length of time, the stimulated ganglion, with its mate of the opposite side, was excised as quickly as possible and the process of fixing and hardening begun. The method from this point on is directed toward having the two ganglia pass through *identical treatment*. *In no instance were they separated from the time they left the animal to the time when, placed side by side upon the same slide, they appeared under the microscope for study.* Not only were they carried through the same reagents, but, *in every case* through the same reagents *in the same bottles or dishes from the first fixing fluid to the solid paraffin.* And further, *the two are cut at the same stroke of the microtome knife, fixed to the slide together, stained together, and appear side by side in the same field of the microscope.*

The carrying of a large number of specimens through the hardening and embedding and cutting processes, keeping each distinct, was greatly facilitated by the following simple device. At first slips of mica were used, but a thin hard cardboard was found to be more convenient. This is cut into strips, — 3×1 cm. is a good size, — and the ganglia, which are carried up to strong alcohol attached to their segment of the cord, are trimmed for cutting and arranged, the two to be compared touching each other, upon one end of the strip of cardboard. A drop of the white of an egg is now placed over them, allowed to dry somewhat, and the whole carefully laid in alcohol. The albumen is

speedily coagulated and holds the ganglia firmly to each other and to the slip. The rule of always placing the stimulated ganglion nearest the end of the slip aids in simplifying matters. Any desired record may be written upon the other end of the slip and all the trouble of keeping a number of little indistinguishable things from becoming mixed up is at once done away with. The cards are, of course, embedded with the ganglia attached. They can easily be removed, if desired, for cutting; but I generally place the specimens so that the plane of cutting shall be parallel to the card. In cutting, it has been my practice to give the face of the paraffin block the shape of a trapezoid, with the stimulated ganglion always toward the shorter side. Each section then carries a record of the arrangement of specimens within it, and any number of sections may be cut and stored, with no danger of confusion. Not only one, but several pairs can be fastened to the same slips arranged in a row so that they may all be cut at the same time. For example, it was my practice to stimulate the right brachial and sciatic plexuses of a frog: this places at our disposal five pairs of ganglia; each pair may be hardened in a different way, and all be arranged as described above on a single slip. They are all cut together, fixed to the slide (by alcohol fixative method), and all stained together. Many slides are obtainable from one such set of ganglia, and each slide may be stained in a different way. Thus, incidentally, a permutation of hardening and staining combinations has been obtained which might form the basis of a separate study.

Not only, in this way, may a dozen specimens be manipulated as easily as one, but they are held in the desired positions relative to each other; and, of special importance, they are cut together. However perfect the microtome, sections do not always come from it of absolutely uniform thickness; and where minute, or even gross, differences of granulation or staining are to be studied, this is of prime importance.

The essential feature, then, of my method is that it compares *corresponding ganglia* of the *same animal* which have been subjected to *identical* treatment in passing from the animal to the slide, *the only point of difference being that one has had its nerve stimulated for a longer or shorter time, while the other has not.* Methods of hardening and staining do not concern us so long as

the two ganglia to be compared go through every step of the process together.

Almost every method has been tried in the hope of obtaining some striking reaction. Some such were found, but up to date they have all proved inconstant. Trzebinski (81) has made a special study of the influence of hardening reagents upon the ganglion cells of the spinal cord. He finds corrosive sublimate one of the best reagents, and states that it does not produce vacuolation of the cell. This method, followed by Gaule's quadruple staining, has given the best preparations for the study of granulation and staining (see Pl. I, Figs. 3-5). Trzebinski, it would seem, did not experiment with osmic acid. This, with hæmatoxylin and safranin, or all four of the Gaule stains, has given a most perfect preservation of the form of the nucleus and the minute structure of the cell protoplasm. Altmann's methods (2) have been tried a number of times, but although beautiful preparations of gland tissues were obtained, nothing definite was brought out in nerve cells.

Two widely different animals, the frog and cat, were purposely selected, upon which to experiment. The results which I will now pass to consider are derived from fifteen experiments upon frogs and eleven upon cats. All the experiments will be referred to either singly or in groups.

Results.

For sake of brevity little more than a tabulation of the results will be given. For further details, see a former paper (24).

Frog No. 1 was given three drops of one per cent curare solution and right sciatic nerve was stimulated continuously for thirty minutes. The three pairs of sciatic ganglia were excised and with those of a control frog hardened in corrosive sublimate. The ninth pair were stained *in toto* in soda carmine, and for some unaccountable reason scarcely any nucleoli could be found in sections of the stimulated ganglion, while they appeared as usual in the ganglion of the other side and in the control ganglia. A count of the two gave the following :—

	nuclei	nucleoli
Six sections of each contained { resting,	122	92
{ stimulated,	177	28

Expressed in per cent, 75 + % of the nuclei in the resting ganglion contained nucleoli to 15 + % in the stimulated. The seventh and eighth pairs, stained in other ways (Kleinenberg's hæmatoxylin and by Weigert's method), gave no such result. In fact, the phenomenon could not be made to reappear in any subsequent experiment.

Next, three similar frogs were taken, each with a control; each was given the same amount of curare and the right sciatic nerves of the three were stimulated continuously one, two, and three hours respectively. From the nine stimulated ganglia no effect of activity could be made out.

Frogs 5 and 6 were used respectively to test the effect of curare and the extent of post-mortem changes in ganglion cells, with results that do not concern us here farther than to say that the use of curare was abandoned¹ and the ganglia were excised as quickly as possible after death. At this point it was also decided to use intermittent instead of continuous stimulation.

Frog No. 7 was made reflex, and the right brachial and sciatic plexuses were stimulated, with two minutes' stimulation alternating with two minutes' rest, for two and a half hours. Marked differences between the cells of the two sides are clearly visible. Perhaps the most pronounced of these, a difference noted independently by a number of observers, is that the nuclei appear shrunken in the stimulated ganglia. This led to the series of measurements summarized in the following table. The nuclei were measured, long and short diameters in sets of one hundred, fifty stimulated and fifty unstimulated being taken from as nearly corresponding sections of the two ganglia as possible. A definite rule precluded wilful selection of the cells to be measured, this rule being that only nuclei containing nucleoli should be measured, and that all such should be taken in the order of their occurrence in the section. Measurements were made with an eye-piece micrometer to the nearest μ under magnification of Leitz oc. 3, obj. 7 (= 600 diameters).

¹ Landois and Sterling, *Physiology*, p. 523, reads: "But when the action of the drug (curare) is fully developed, no amount of stimulation of the skin or the posterior roots of the nerves will give rise to a reflex act, although the motor nerve of the ligatured limb is known to be excitable." My experiment on frog 5, in which *all but the sciatic nerve, bone and all, was severed*, gave exactly the above result. The reason for absence of results, however, in my case, may have been continuous stimulation or curare or both.

TABLE I.

Frog No. 7, made reflex. Stimulated two and one-half hours, intervals of rest and stimulation being two minutes. Three sets of one hundred nuclei each.

		Nuclei in μ mean diameters.			Cells in μ mean diameters.
Ganglia hardened in cor- rosive sublimate.	8th pair.	Resting.....	13.57	Set 1.	39.69 35.00
		Stimulated.....	12.23		
		Diff.....	1.34		
	9th pair.	Resting.....	13.94	Set 2.	
		Stimulated.....	12.56		
		Diff.....	1.38		
	2d pair.	Resting.....	14.48	Set 3.	
		Stimulated.....	13.26		
		Diff.....	1.22		
	Sets 1, 2, and 3, volume shrinkage, 24 %. ¹				

The five succeeding experiments were made with the purpose of getting the greatest possible amount of change; and under the supposition that this might be obtained, for the frog at least, in shorter time, if the nutrition of the cells was prevented, the frogs were thoroughly bled or the capsules of the ganglia torn off. None of these experiments gave definite results. Sections of both ganglia appear, stained by Gaule's method, redder than normal, indicating apparently a clogging of the cells with decomposition products. Stimulated and resting show alike vacuolation, perhaps the same as that observed by Rosenbach (68) in starving dogs. The nuclei in both are shrunken, but show no marked difference in size.

Results of a single experiment of this class need be given.

TABLE II.

Frog No. 8, bled. Stimulated for seven hours, five minutes of stimulation alternating with five minutes rest. One set of one hundred nuclei.

		Mean diameters in μ .	
Ganglia of 8th pair, hardened in corrosive sublimate and stained by the Gaule method.	Resting.....	12.36	Volume shrinkage, 8 %.
	Stimulated.....	12.01	

¹ The volume shrinkage per cent is computed from the mean diameters, treating the nuclei as spheres.

One experiment, in which the ganglia were suspended in a large beaker of sterilized normal salt solution, gave more definite results.

TABLE III.

Frog No. 14. Sciatic ganglia of right side suspended in salt solution and stimulated three and one-half hours, five stimuli per second, one minute of stimulation alternating with one minute of rest. The ganglia of left side kept during this time in blood of same frog. Two sets of one hundred nuclei each.

		Mean diameters in μ .	
9th ganglia. Corrosive sublimate, with Gaule's stain.	Resting.....	14.70	} SET 1.—Measured by myself <i>previous</i> to Mr. W.'s measurement of set 2.
	Stimulated..	13.10	
	Diff.....	1.60	
	Resting.....	14.57	} SET 2.—Measured by Mr. W. <i>without knowl-</i> <i>edge of my results</i> , and having but one of the ganglia in the field at the same time, and <i>not knowing which had been stimulated</i> <i>and which not.</i>
	Stimulated..	12.14	
	Diff.....	2.43	

Sets 1 and 2, volume shrinkage, 36 %.

Thinking that greater changes might be obtained at a higher temperature, one experiment was made to test this; and, though not entirely successful, the result may be given.

TABLE IV.

Frog No. 15. Cerebrum removed, and wound allowed to heal before experiment. Stimulated five and one-half hours at temperature 35° C.; intervals of rest and stimulation, one minute. Two sets of one hundred nuclei each.

		Mean diameters in μ .			
Ganglia of	2d pair. Hardened in picric acid. Gaule's stain.	Resting.....	16.53	} SET 1.—Measured by myself <i>previous</i> to measurement of set 2.	
		Stimulated....	15.66		
		Diff.....	.87		
			Resting.....	17.40	} SET 2.—Measured by Mr. L. without knowledge of my own measurements, and <i>not knowing which of the ganglia</i> <i>had been stimulated.</i>
			Stimulated....	15.84	
			Diff.....	1.56	
	9th pair. Fleming. Gaule's stain.		Resting.....	20.90	} SET 3.
			Stimulated....	19.13	
			Diff.....	1.77	

Sets 1, 2, and 3, volume shrinkage, 12.5 %.

It may be noted that both Mr. W.'s and Mr. L.'s measurements make the difference greater than my own. Staining and structure of protoplasm not well defined; due probably to the fact that the frog died toward end of experiment. At its close the muscles were beginning to pass into *rigor mortis*.

It was thought desirable at this stage to ascertain whether the results above detailed for frogs hold good for a mammal. So far, experiments have shown that the most marked results are to be obtained by keeping the animal in the most normal condition. Functional activity of the nerve cells of a mammal can certainly not be studied many seconds after the circulation is stopped; whereas an animal is active for hours at a time, and the experiments, if success is to be attained, must be continued for a similar time. I think I am justified in distrusting the influence of curare even upon the central portion of the reflex arc. Narcotics and anæsthetics, although they do not stop the cardiac and respiratory movements, if given in proper amount, produce most profound changes in the activity of nerve centres. So far as known, they may or they may not cause correspondingly marked histological changes in the nerve cells. However this may be, it was determined to run no risk of complicating matters by their use, and accordingly a method of producing insensibility without the use of drugs was resorted to.

The cat was chosen for farther experiment. The method¹ of procedure is briefly as follows: The cat is laid on a holder and gently brought under the influence of ether. When fully anæsthetized, the skull is trephined at about the parietal eminence, and a slit made through the dura, care being taken to dodge any blood-vessels which may be in the neighborhood. The trephine used was about 5 mm. in diameter. With kittens it is possible to lift out a small piece of bone with the point of a knife-blade with generally less loss of blood than is occasioned by trephining. Now, holding the head with the left hand, the thumb upon the vertex, the tip of the first finger upon the angle of the right jaw, the tip of the third finger upon that of the left jaw, introduce, through the opening in the skull, the blunt end of a 3 mm. glass rod, and aim it directly at the angle

¹ This method was obtained from a paper entitled "On the renal circulation during fever" (Walter Mendelson, *Amer. Jour. Med. Sci.*, Phila., 1883), where the method is credited to Ludwig.

of the right lower jaw, the opening being invariably made in the left parietal bone. The probe will strike the floor of the skull, having pierced the right optic thalamus and the right crus. Work the probe across the floor of the skull about 3 mm. to either side of its first position and withdraw it. Introducing the probe again, direct it forward as before, but directly ventral, aiming to pierce the left optic thalamus and left crus. Take about one 3 mm. step with the end of the probe to right and left, withdraw probe, and close the skin over the wound. The purpose of the operation is, of course, to destroy sensibility in the cerebrum and to cut off the sensory and motor tracts in the crura; and if successful, complete anæsthesia, with normal pulse and respiration, should result. Remove the ether immediately and allow the animal to recover. It should show no signs of pain or distress, but should remain as though sleeping quietly during the rest of the experiment. In some cases, however, the animal does become restless for a few minutes after the ether passes off. This condition generally lasts but a short time and gives place to the state of quiet sleep desired. After this treatment stimulation may go on for any reasonable length of time with no further trouble. I can recommend the method to any who wish to make prolonged experiments not involving the returning of the animal to consciousness. It is not, of course, always successful. In some cases, the respiratory centre becomes involved, spasmodic gasping sets in, and unless artificial respiration be employed, the experiment is at an end. Examination has shown that this is due generally to hemorrhage spreading downward from the section in the crura into the substance of the medulla or between the medulla and floor of the skull. Consequently the probe should be so manipulated as to injure the blood-vessels in the floor of the skull as little as possible.

The next step is to get the electrodes over the desired nerves; and, throughout the experiments, the nerves of the right brachial plexus were employed. Turning the animal upon its back, expose the external pectoral muscles by an incision through the skin about two inches long midway between the sternum and axilla. Cutting now through the pectoral muscles will expose the subclavian artery and vein, and just underneath these can be seen the nerves of the brachial plexus. In order to pre-

vent hemorrhage, I always take the muscles up with a double row of ligatures and make the cut between them. Carefully free the plexus from fat for a short distance and, without injury to the nerves or blood-vessels going to them, separate them from the subclavian vessels, and, not including these, slip over the plexus from behind a two-tined platinum electrode.¹ Thus the current is made to pass through the nerves obliquely. By including the whole plexus at this point, four ganglia are stimulated. As in the frog experiments, the nerves are not divided, and as the stimulation begins, every muscle of the right leg should contract. This is, in fact, the test of the proper working of the experiment.

The animal is now to be carefully tended while the stimulation proceeds. The temperature is frequently taken and heat applied or withdrawn as the case demands. Respiration and pulse are watched. Lymph is apt to collect in the axilla about the electrodes and should be frequently wiped up with absorbent cotton. With the electrodes in place, the skin is drawn together over the wound and held with a clamp, and the wound is further protected by an ample pad of cotton. In my experiments, strictly antiseptic precautions were not taken. All tools, however, which touched either the wound in the head or axilla were sterilized before each operation; and, in no case, did any perceptible inflammation make its appearance. As before, the mate ganglia of the left side were in all cases used as control. A double control was employed at first, consisting of a pair of thoracic ganglia from the same animal carried through with each pair of test ganglia. This was soon found to be unnecessary, since the cells of these control ganglia resembled those of the resting ganglion. The results of the first experiment may be read from the following table:—

¹ The electrode first used was the platinum-tipped electrode ordinarily used to stimulate muscle-nerve preparations. Thinking that it would be better to have the platinum tips guarded, I made an electrode by letting heavy copper wires into deep saw grooves in a strip of gutta-percha. The platinum wires were soldered to these and were made to lie half-exposed in shallow grooves upon the inner side of each of two fork-like prolongations of the gutta-percha.

TABLE V.

Cat No. 1. Stimulated for seven hours ; intervals one minute, spaced by hand.

		NUCLEI (4 sets, 100 each).		CELLS.
		Mean diameters in μ .	Shrinkage in volume.	Mean diameters in μ .
Ganglia of	1st thoracic. Hardened in osmic acid.	Resting....	16.29	59.06
		Stimulated..	14.07	57.19
		Diff.....	2.22	
	8th cervical. Hardened in corrosive sublimate.	Resting....	14.91	(Selected.) All over
		Stimulated..	11.70	51 % (T.) 50 μ .
		Diff.....	3.21	
	7th cervical. Hardened in Flemming's fluid.	Resting....	16.60	57.50
		Stimulated..	15.41	56.25
		Diff.....	1.19	
	6th cervical. Hardened in picric acid.	Resting....	14.98	44.21
		Stimulated..	14.23	14.6 % 44.74
		Diff.....	.75	

Sets 1, 2, 3, 4, volume shrinkage, 28.6 %.

Several points in the above table call for remark. The seat of most active change is again seen to be within the nucleus. It is to be noted also that the greatest amount of difference between resting and stimulated nuclei occurs in the 1st thoracic and 8th cervical ganglia. This may be due to the fact that a greater number of the nerves from the 6th and 7th cervical ganglia escape stimulation. Or it may be that, coming first between the electrodes, the branches from the 1st thoracic and 8th cervical tend to short circuit the current and thus deprive the others of a due share of the stimulation. At any rate, the 6th and 7th cervical have failed to show the effect of stimulation to the extent shown by the 8th cervical and 1st thoracic; and for clearest results I have found it best to include in the circuit the medius and spiralis nerves, with the small branches lying between and behind these, and then use only the 8th cervical and 1st thoracic ganglia. Another word of explanation may be added. It must be taken into account that, in claspings the whole plexus between the tines of the electrode, we are stimulating an enormous number of nerves. When the strength of the stimulation is tested, if the tip of the electrode only is touched to the tongue, the stimulation is concentrated on a small area and affects but a few nerve fibres. The stimulation

is hence felt to be severe ; whereas if the electrodes are laid full length upon the tongue, the stimulus can scarcely be felt at all. The neglect of this fact at first has resulted in the use of quite inadequate stimulation.

Stimulation in this case was, however, severe. It was frequently increased by sliding up the secondary coil, and was so regulated as to produce the greatest possible amount of muscular contraction in the right fore leg without causing reflex contractions in other parts of the body. Contractions in this leg toward close of experiment were feeble but constant. Within five minutes after the animal was bled, the muscles of this leg had passed into *rigor mortis*, the muscles of all the other limbs being normal and irritable. Pulse and respiration remained normal the whole time.

Aside from shrinkage of the nuclei, other important changes occur. For the 1st thoracic pair, hardened in osmic acid, the nuclei are plump and round in the resting ganglion, and stain lighter than the cell protoplasm. In the stimulated ganglion they are irregular in outline and stain much darker than the rest of the cell. This appearance is due not only to a darker staining of the nucleus, but to a lighter staining of the cell. Holding the osmic acid sections of resting and stimulated ganglia over a white surface, it is not difficult to see with the unaided eye that the resting ganglion is stained darker than the other. This indicates that a substance which reduces osmic acid has been used up or changed, in the stimulated cell, into something which does not reduce the acid ; while in the nucleus more of something which reduces osmic acid has been produced during stimulation. Examined microscopically, the lighter stain is seen to be due to extreme vacuolation of the cell protoplasm. This does not occur in the resting ganglion of the left side or in the two thoracic ganglia used as control. The general appearance is well shown in Figs. 1 and 2 of Pl. VII ; although the vacuolation of the cell protoplasm in Fig. 2 has not been well copied from the original drawing. The protoplasm of all the cells shows definite vacuolation to a greater or less extent.¹ It was also noticed *independently* by three² observers that the

¹ This appearance is better represented in Figs. 3 and 4, *Amer. Jour. Psy.*, Vol. II, p. 403.

² The three were Dr. H. H. Donaldson, Dr. Wm. H. Welch, and the author.

nuclei of the cell capsule were shrunken in the stimulated ganglion. This may be seen by comparison of Figs. 2, 4, and 5 with Figs. 1 and 3 of Pl. VII, and holds good also for diurnal fatigue, for which compare the capsular nuclei of Figs. 7 and 6, Pl. VIII. This may indicate the supposed nutritive function of the capsular cells.

The 8th cervical ganglia, hardened in corrosive sublimate, show for the right ganglion the shrunken and darkly stained nuclei characteristic of stimulation (compare nuclei in Figs. 3 and 4, Pl. VII). The vacuolation of the protoplasm is not brought out, although well preserved by the same method in some of the frog's ganglia. Flemming's fluid and picric acid happened to be used here by way of experiment, but were found to give, on the whole, inferior results.

TABLE VI.

Cat No. 2. Stimulated one hour forty minutes; intervals one minutes.

		100 NUCLEI. Mean diameters in μ	Shrinkage in volume.	100 CELLS. Mean diameters in μ .
Ganglia 1st thoracic. Osmic acid.	{ Resting.....	14.91		48.10
	{ Stimulated....	13.51	25.6 %	46.53
	Diff.....	1.40		

Examination of sections shows similar changes to those described for cat No. 1, but much less in degree.

No attempt was made to render the stimulation equal in the two experiments; but it is strongly hinted by the results that a quantitative relation exists between the amount of stimulation and amount of change in the cells. Such a relation should obtain, if we are dealing with physiological cause and effect. To test the point with mathematical precision is, of course, impossible; for we must know, in order to do this, not only the strength of stimulus used, but also that the same amount of stimulus is distributed to the same number of cells; and, further, that the ganglion cells of one animal are exactly as irritable as those of another animal. However, to decide the matter, a series of experiments was arranged under the assumption that the irritability of cats is in general the same, and that the same nerves in different cats connect approximately with the same number of ganglion cells. To make these factors as nearly alike as pos-

sible, half-grown kittens were used throughout. The strength of stimuli was regulated by placing a rheocord, resistance-box, and galvanometer in the primary circuit derived from two 1 l. copper sulphate cells. By manipulation of the resistance-box and rheocord, the galvanometer needle was brought to a given position and held at this point during the whole of each experiment. The experiments were made in rapid succession and without altering the setting of the apparatus. Stimuli were purposely not severe, because of the long duration of some of the experiments. But not until the series had been studied was it clear that the stimulation was too slight for the most definite results.

TABLE VII.
SERIES WITH EQUAL STIMULATION.

Intervals of 15 seconds' stimulation, alternating with 45 seconds' rest. Ganglia of 1st thoracic pair, hardened in osmic acid.	CAT No. 7 (operated upon and left without stimulation 2½ hrs.).	Length of stimulation.	No. of nuclei measured.	Mean diameters in μ .	Shrinkage in volume of nucleus.
		0 hrs.	100	14.20 14.54	¹ - 6.9 %
		1 "	100	14.70 13.51	+ 22 %
		2½ "	200 (T.) ²	11.86 10.95	+ 21 %
		5 "	100	15.97 14.38	+ 24.3 %
		10 "	100		
			³ S. 100 S. 100 (T.) 100 (T.)	16.19 13.35	 + 43.9 %

Two experiments were made to test the effect of stronger stimulation and the influence of the rest interval, with the suggestive result expressed by Table VIII.

¹ The minus sign indicates that the nuclei of the right side are slightly larger in this case. In the only other set measured from a normal pair, the nuclei were also a little larger on the right side.

² Sets marked "T." are measured by a third person, with whom every precaution was taken to obtain purely mechanical and unprejudiced measurements.

³ Sets marked "S." (selected) are those in which only nuclei in cells of over 50 μ diameter were measured. The shrinkage volume per cent is given for the two unselected sets, not marked "S.," and are thus comparable with other members of the series. The shrinkage of the selected sets is 49.9 %.

TABLE VIII.

STRONGER STIMULATION AND SHORTER REST INTERVAL.

	Time.	No. of nuclei measured.	Mean diameter.	Volume shrinkage.
1st thoracic ganglia. Osmic acid.	CAT No. 9 (45 seconds' rest to 15 seconds' work).... 2 hrs.			
		100	12.39	
			10.45	40.9 %
	CAT No. 10 (intervals 15 seconds' rest to 15 sec- onds' work)..... 2 "			
		100 (T.)	13.82	
			12.04	32.7 %

Two facts are apparent. First, with stronger stimulation, naturally enough, the effect may be produced in two hours that, with slight stimuli, it required ten hours to obtain. Second, the length of rest intervals is of great importance. Although No. 10 received exactly twice as much stimulation in the two hours, the cells show considerably less change than those of No. 9.

Stimulation has brought out a functional differentiation of some sort between the large and small cells of the spinal ganglia. The large cells show the effects of work; the small cells very little, or not at all. The fact is too well marked to pass by unnoticed.

Considering all cells large which have one diameter 50μ , or over, and those small which have not, a count gives the following result:—

CAT No. 11. — FIRST THORACIC GANGLIA.

	In 100 large cells nuclei.		In 100 small cells nuclei.	
	Shrunken.	Not shrunken.	Shrunken.	Not shrunken.
Resting.....	5	95	0	100
Stimulated.....	94	6	8	92

A few fibres going to a ganglion, the vertebral branch, etc., escape stimulation by our method. This accounts for the few large cells which do not appear worked in the stimulated ganglion. It cannot account for the multitude of small cells, comprising numerically more than half the cells of the ganglion, which do not show the effects of stimulation. After some searching, a field was found (Pl. VII, Fig. 2) in which every nucleus was shrunken; but I am now free to confess that only short-sighted judgment led to its selection for the plate. No difference between the cells other than size is discernible.

Regrets come always too late; and so, only after the work had been done, the long, tedious measurements completed, and the results footed up, did I notice how widely, in point of size, the cells of one cat differ from those of another (compare cats 6 and 11), and wish that I had weighed the cats. Cats 6 and 9 were females, and small. However, the question as to whether the size of animals of a species differ by the size or number of cellular elements, or both, is not entirely germane to our subject. Gaule¹ would maintain that for any species the number of cells is a constant, variations of size to be accounted for by size of cells. Such a wide variation in the size of cells as is here seen favors this view.

Many devices were employed to eliminate the personal equation and obtain mechanical measurements. Three persons unacquainted with the methods of the research kindly consented to assist in the work of measuring. Even here the differences are too plain to make an absolutely neutral state of mind long possible, since each of the three, before completing the measurement of a single set, had noticed that the nuclei in the two ganglia were different. In my own measurements, I was wont, from the first, to throw all thought of the work as completely as possible off my mind, to think about something else, to have an interesting story read aloud. In general, also, all the measurements of a series were made before any results were footed up, so that the way they were tending could have no unconscious influence.

This laborious and time-consuming method of treating the sections has been adopted in order to gain some slight mathematical hold directly upon the working of the nerve cell. It is, however, inadequate to express the facts of the case, and it is at best but a poor expression of the amount of change. In the first place, it is impossible to measure accurately the jagged outline of a worked nucleus. Our practice has been to measure well out toward the tips of the irregular points into which the nucleus is prolonged; and this would tend, evidently, to make the computed, larger than the actual volume. In the second place, the quantities in the tables are *averages*; whereas, for

¹ GAULE, JUSTUS, Zahl und Verteilung der Markhaltigen Fasern im Froschrückenmark. Abhandl. d. Math.-phys. cl. d. k. Sächs. Gesellsch. d. Wissensch., Bd. XV., No. 9, pp. 739-780. 1889, Leipzig.

our study, *extremes* are naturally of more interest. The ideal would be to follow a living nerve cell during stimulation from the normal resting state to the condition of extreme fatigue. This I have not succeeded in doing to entire satisfaction as yet. But it is possible, in the study of a section, to find a fairly good substitute for the ideal. We see some cells which are not affected at all; and this we should expect, because it is impossible to stimulate all the fibres going to a ganglion without cutting so close as to endanger its blood supply. Next, we find cells that are slightly worked. In the even outline of their nuclei there may be here and there a slight indentation, and the nucleus may stain a shade darker; now and then a small vacuole makes its appearance in the cell protoplasm. These nuclei may have shrunk five or ten per cent. And so we pass, by all degrees of difference, to cells which show extreme fatigue. And here the protoplasm is riddled with vacuoles, and the nucleus has shrunk to a densely staining speck, which must have lost seventy-five to eighty per cent of its original volume.

I may close this section with the concluding sentences of a former paper. We have, then, "as a result of electrical stimulation:—

"A. For the nucleus: 1. Marked decrease in size. 2. Change from a smooth and rounded to a jagged, irregular outline. 3. Loss of open reticular appearance with darker stain.

"B. For the cell protoplasm: 1. Slight shrinkage in size. 2. Lessened power to stain or to reduce osmic acid. 3. Vacuolation.

"C. For the cell capsule: Decrease in size of the nuclei" (24, p. 397).

V. PROCESS OF RECOVERY FROM FATIGUE.

At the point in the investigation reached at the close of the last section, the objection was raised that just such appearances had long been known to occur in pathological conditions of the nervous system. It is true that they resemble changes hitherto described as pathological; but up to the present no attempt has been made to distinguish changes due to fatigue from those caused by disease, and on *a priori* grounds we should expect

the phenomena of fatigue to precede and shade into those of disease.

Several facts connected with the research negative the objection; none, not even the so-called pathological appearance of the cells, give it any real support. In the first place, no pathological factor, capable of affecting the spinal ganglia, has been introduced into the experiments. Electrical stimulation is kept within physiological limits, as shown by the fact, that the nerves conduct impulses to their muscles throughout the experiment. And most of all, the fact, that the change increases steadily in amount, as stimulation is prolonged or intensified, would indicate that we are dealing with normal processes of the active living cell.

But aside from considerations of a pathological nature, the process of recovery in a tissue has an interest of its own, physiological and hygienic, in no degree less than that which attaches to the process of fatigue itself. The bearing of the literature upon this point has already been discussed. We know that the cells of the epidermis, from which the nerve cells are phylogenetically derived, are worn out and off and are replaced by new cells produced by multiplication. This is doubtless true of all stratified epithelia, lining surfaces, internal as well as external. But in all these instances we have friction and contact with foreign or irritating substances, the half-masticated food forced through the narrow œsophagus, dry air passing rapidly in the trachea, and urine in ureters and bladder. Friction we have in the blood-vessels; but who has ever found epithelial scales in the blood such as occur in urine or in saliva?

From what is known of the structure and development of the nervous system, the gradual growth of the nerve fibre from the cell, the length of time required for the regeneration of a divided nerve, the lack of any evidence of fatigue in a nerve, etc., it would seem as absurd to suppose that the nerve elements die out and are replaced as to advocate the daily destruction and rebuilding of the world's telegraphic systems. Cables and wires and keys, accidents aside, are practically permanent; and so are the battery cells, the zincs and acids alone requiring renewal. So that if it were proven that, after stimulation, the cells of a spinal ganglion fail to recover, *i.e.* die out, and are replaced by new cells, I should be free to admit that a pathologi-

cal condition had been induced, rather than suppose that this were the normal daily procedure.

To study recovery, then, it was arranged to stimulate a series of cats, equally and for the same period, and then allow members of the series to rest for different lengths of time.

The most perfect apparatus for controlling stimulation were supplied me by the physical and physiological laboratories of Clark University. A Weston's direct reading ammeter, reading from 0-15 ampères, was placed next the battery. From this it was possible to read off the strength of current at any moment. Next to this in the circuit was placed a resistance-box with rheocord attached. This is necessary for exact work, as the battery was set fresh at the beginning of each experiment and increased in power for the first hour or so, and then gradually weakened until the end of five hours, during which the stimulation lasted. These variations could generally be compensated for by sliding the bridge of the rheocord.

It was decided to use twenty stimuli per second, and this rate was obtained by loading the interrupting hammer attached to the induction coil. To make certain that this did not jar out of adjustment, I was compelled to place also in the primary circuit a signal which should write its vibrations under the tracing of a signal in circuit with a seconds clock.

The same interval was adopted as for the last series, viz. forty-five seconds' rest alternating with fifteen seconds' stimulation. I am indebted to Dr. Lombard for the most inexpensive and serviceable little device ever invented for the spacing of intervals. A small nickel clock forms the motor part of the contrivance. It must be provided with a second hand. The glass face-cover and all the hands are removed, and upon the shaft of the second hand is fastened an eccentric zinc disc $2\frac{1}{2} \times 3$ cm. in diameter. In front of the clock is held by a post, properly placed, a lever of hard rubber 15 cm. in length; the longer arm of the lever, 8 cm., is between the post and the clock, so that this end, which is tipped with a small gutta-percha wheel, to reduce friction, will tilt back lightly upon the eccentric. The other arm of the lever carries two light copper wires tipped with platinum. The platinum tips, extending downward at right angles from the lever, dip into a glass mercury-cup. Thus the motion of the eccentric upon the second shaft is made to tilt the lever in and out of the

mercury every minute. By placing the cup upon the head of a screw, so that the mercury can be raised and lowered at will, and by proper shaping of the eccentric disc, it is easy to so adjust it that the circuit is made through the mercury fifteen seconds and broken forty-five seconds, which is the spacing of intervals desired. The whole is arranged upon a small board, into which two binding screws are set for convenience of joining up with the circuit. This is, of course, placed in the primary circuit.

By this arrangement it was possible to control stimulation quite accurately. A half ampère, as read from the galvanometer, was used throughout the series. The automatic make and break key gave regular intervals of rest and stimulation. The beat of the interrupter was kept at twenty per second. The secondary coil was set at a certain place and moved up at regular intervals, in the same manner for all the experiments.

The animals used were a lucky lot, five gray kittens six or eight weeks old, and as much alike as peas in a pod. Nothing was fed after the commencement of the experiment, but up to that time they were so well fed that it was thought a fast of eleven to thirty hours would not complicate matters seriously, if at all. The operation was made in every respect according to the description already given. Stimulation was continued for five hours in each case. The animal was then gently removed from the holder, wrapped up, and laid in a warm place, where it was left to sleep the desired number of hours. At the expiration of this time the ganglia were cut out as quickly as possible. The 1st thoracic and 8th cervical pairs were used and were hardened respectively in one per cent osmic acid and saturated mercuric chloride solution of 40°C. The precautions regarding exactly similar treatment were the same as described for the preceding series, and the cells and nuclei were measured and dealt with by the method of arithmetical means as before.

As there is often reason to distrust averages, I will give the actual figures as they occur in a sample sheet of my notes taken at random. The measurements were made with a Zeiss eye-piece micrometer ruled to $\frac{1}{4}$ micron divisions (eye-piece 8; objective 4.0 mm. \times 500), hence each division equals $2\frac{3}{8}\mu$. They are given, as read, in units of the micrometer.

CAT NO. 17. — MEASUREMENT OF DIAMETER OF NUCLEI.

<i>Right.</i> — After 5 hrs. stimulation and 0 hrs. rest.		<i>Left.</i> — Normal.	
Diameter.	Number of measurements.	Diameter.	Number of measurements.
8.5.....	3	9.	3
8.	1	8.5.....	6
7.5.....	4	8.	28
7.	17	7.5.....	17
6.5.....	17	<u>7.</u>	<u>61</u>
6.	48	6.5.....	29
<u>5.5.....</u>	<u>30</u>	6.	33
5.	44	5.5.....	12
4.5.....	14	5.	11
4.	17		200
3.5.....	4		
3.	1		
	200		
Average diameter for set, 5.39.		Average diameter for set, 6.83.	

The above is sufficient to show that the mean of these diameters is a fair average. The measurements stand in about equal numbers above and below it in both cases. The largest nuclei are found among the normal cells and the smallest among the stimulated cells.

The results of the whole series may be seen at a glance from the following table : —

TABLE IX.

SERIES TO SHOW INFLUENCE OF REST.

Right brachial plexus of each stimulated in the same manner for five hours, and allowed to rest.

NUCLEI.				CELLS.
	Rest.	Mean diameter of nucleus in μ .	Shrinkage.	Mean diam. in μ .
CAT 17.....	0 hrs.	16.40 Left, <i>normal</i> . 12.93 Right, <i>stimulated</i> .	48.8 %	57 52
CAT 16.....	6.5 hrs.	16.70 Left, <i>normal</i> . 15.09 Right, <i>stimulated</i> .	26 %	56 54
CAT 21.....	12 hrs.	16.34 Left, <i>normal</i> . 14.73 Right, <i>stimulated</i> .	26 %	55 51
CAT 19.....	18 hrs.	17.08 Left, <i>normal</i> . 16.03 Right, <i>stimulated</i> .	18 %	56 55
CAT 18.....	24 hrs.	17.01 Left, <i>normal</i> . 17.11 Right, <i>stimulated</i> .	+ 2 %	
From Table VII.				
CAT 7.....	Normal.	14.20 Left. 14.54 Right.	+ 6.9 %	

In this series stimulation was severe ; but it must be remembered that during the so-called five hour period of work it was applied for only fifteen seconds each minute. Five hours of stimulation represent, therefore, only one hour and a quarter active working of the cells. In this short time the change is marked as shown by a shrinkage of 48.8 per cent in the nuclei of the stimulated side. The cells, as before, shrink little, and the cell protoplasm exhibits considerable vacuolation. (For effect of five hours' stimulation, cat 17, compare Fig. 1 with Fig. 2, and Fig. 3 with Fig. 4. For influence of rest, cats 16 and 17, compare Fig. 5 with Fig. 4 and with Fig. 3, Pl. VII.) This, as before remarked, is not well shown in the plate. In general, substance is lost from the cell interstitially as shown by vacuoles and lighter granulation, while the nucleus collapses bodily. This would seem to indicate that the reticulum of the cell protoplasm is stiff and elastic enough to hold its shape when the

interfibrillar substance is removed, whereas that of the nucleus is too soft or delicate to resist the pressure of the lymph about it.

The ideal, in following the process of recovery in a nerve cell, would be to watch continuously a living active cell for the required length of time. For the present, however, we have only specimens prepared by two good methods, taken so as to give us presumably five steps in the process.

As before remarked, the table gives but a meagre notion of the facts. The processes of recovery are, in general, the reverse of those of fatigue. The nucleus and protoplasm gradually return to normal appearance. The protoplasm seems to recover rapidly. At any rate, in the specimen which has rested six and one-half hours, little trace of vacuolation is observable; and this is true of all those which have rested for a longer time. The nuclei, on the other hand, recover slowly. After six and one-half hours' rest they show a marked gain in size, but still retain the dense stain characteristic of fatigue. Indeed, in this respect the process of recovery is not entirely completed in all the nuclei which have rested for twenty-four hours, it being still possible to find a few large but densely stained nuclei. So far as it goes, my observations, therefore, favor the view that granules arise within the nucleus in some peculiar manner, although in a nerve cell they are too small and ill-defined by any method I have used to permit of seeing the manner of their migration into the cell protoplasm, if, indeed, any such thing takes place.

A study of nerve cells, thus, after long periods of complete rest, has brought out a point of general interest to the histology of the nervous system. An appearance often noted in nerve histology has hitherto complicated all of our experiments. This is the fact that individual cells in the same ganglion present such great histological differences. Ranvier¹ calls attention to this fact and proves that it cannot be due to the action of reagents, but must express some difference between the cells themselves.

¹ RANVIER, *Traité D'Histologie*, Paris, 1889, p. 802: "How is it that a little ganglion, placed in a solution of ammonium bichromate, all the elements of which are therefore submitted to the same influences, contains, side by side, cells modified in a manner so widely different? This is a fact which we cannot yet explain, but upon which we must insist, because we see it repeated in the spinal cord, the cerebrum, the cerebellum, etc.; that is to say, in all organs containing ganglion cells."

In my own experiments, even in sections of normal, resting ganglia, I invariably find a few cells which have all the appearances of being worked. The number of these cells in normal ganglia varies, but may reach five to ten per cent, while in stimulated ganglia they often exceed ninety per cent. My theory was in such cases that some of the cells had become more or less fatigued by the ordinary activity of the animal. This was merely supposition. It might also have been supposed that these cells were in process of degeneration. But after we have wrapped up an animal in cotton batting and laid it in a warm chamber at constant temperature for twenty-four hours, its brain having been previously destroyed, so that it makes no voluntary movements, after scarcely a sensory impulse has broken the rest of the cells for that length of time, we find, as might be expected, all the cells in most perfect resting condition. The cells appear uniformly full, with not a single shrunken nucleus visible. The nuclei, in fact, appear larger, rounder, and clearer than in any specimen I have hitherto examined. It would seem, therefore, quite possible that the differences between ganglion cells, observed in sections of the same specimen, may be due to the phase of functional activity or of nutrition in which each of the cells happened to be when it died or was killed by the reagent.

No one is better aware than the writer that repetition of such a series of experiments is desirable. My time and work, however, did not permit of this; and it was thought preferable that some one else should be allowed to make the repetition, in case these experiments are not considered conclusive. Everything in the work has been made as exact and mathematical as possible, on the one hand, in order to do away with the necessity for repetition, and, on the other, to make exact repetition possible.

As far as the specimens obtained from the series are concerned, they leave no room for questioning the two following conclusions:—

First, that spinal ganglion cells of kittens do recover from the effects of electrically stimulating the nerve going to them.

Second, that recovery may be a slow process. It is not complete after eighteen hours, but is found to be about complete after a rest of twenty-four hours.

Pre-eminently master among the tissues of the animal body, controlling their activity in so many ways, in starvation holding

its own by the tribute rendered to it even by muscle, I had expected to find the power to recover much more energetic in the nerve cell than in gland cells, the process of recovery in which has received some attention (on this point see 39, p. 587). No attempt to draw any exact time parallel between the action of the gastric cells of a frog and the spinal ganglion cells of a kitten is to be understood from the present reference. It is, however, of interest to note in this connection that Langley and Sewall found that, upon feeding a frog, the granules commenced to pass out of the cells of the stomach, and continued to do so for about six hours, when they began to fill up the cells again, and recovery was not complete until twenty-four hours had elapsed (34, p. 676). That is, to recover from six hours' secretion required twenty-four hours' rest.¹

VI. CURVE OF NERVE CELL FATIGUE AND RECOVERY.

In the foregoing, data are present from which to construct a curve that may provisionally, at least, be taken to represent the process of fatigue and recovery in the cells of the spinal ganglia. Whether these results are applicable to the action of other kinds of nerve cells, it is impossible to say with certainty. And whether the action of the nucleus may be fairly considered an index of the whole process is open to question. But we have shown that this shrinkage of the nucleus is directly proportional to the duration and also to the intensity of stimulation, and, in general, inversely proportional to the length of the period of rest. At any rate, it is the only index we have at present, and we may be permitted to use it with the understanding that the curve obtained is entirely provisional.

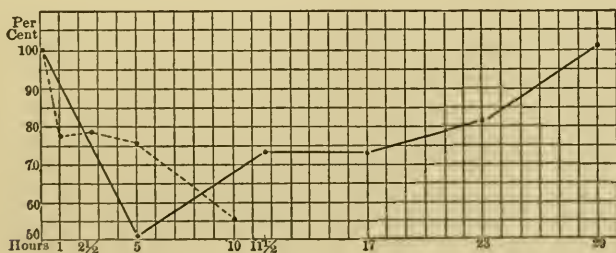
The curve of fatigue for a muscle is generally described as a straight line which falls more or less abruptly according to its load, and the strength and frequency of stimuli applied to it. The fatigue of a muscle *in situ* is, moreover, an exceedingly slow process (39, p. 547), if a physiological process at all. Roth stimulated muscles continuously for as long as twenty days

¹ It will be remembered that, if a frog is fed a piece of sponge instead of a worm, recovery may be greatly slowed. In another series of experiments I shall attempt feeding regularly. However, Langley's experiments and my own in this respect are clearly not comparable, since sponge acted to produce a much longer stimulation than food.

before producing complete exhaustion. The fatigue curve of a nerve fibre has been shown, for short intervals at least, to be a straight line which remains parallel to its base line; *i.e.* within physiological limits a nerve fibre is not susceptible of fatigue (8; 9; 79; 40).

No curve representing fatigue of the nerve cell, drawn directly from observation of the cell itself, has hitherto been made. The nearest approach to this is to be found in such work as Mosso (54, pp. 175, 185, 186) and Lombard (44, Figs. 3 and 5) have done for the fatigue which manifests itself in voluntary muscular contractions (Mosso, see plates pp. 178, 185, 186; Lombard, see Pl. II, Fig. 5). If, as would seem demonstrated, the curve which these investigators find, expresses, in some way, the fatigue of the brain or spinal cord cells, we may say that the nerve cell tires rapidly at first, then slowly, or possibly gains a little or holds its own for some time, and at last falls quite rapidly again to a state of complete exhaustion. It is not possible, of course, to say whether any nerve cell, even the most shrunken and vacuolated to be found, has been entirely exhausted; probably not; so the end of our curve will not be complete. But if we now plot the percentages given in table VII for a fatigue series, we find a curve quite similar to those obtained by Mosso and Lombard.

We have from the table slight stimulation, for one hour, two and one-half hours, five hours, and ten hours, causing a shrink-



age in the volume of the cell nucleus of respectively 22 per cent, 21 per cent, 24.3 per cent, and 43.9 per cent. This is represented to the eye by the dotted line in Fig. 1. For the first hour the nuclei shrink rapidly, for three or four hours they almost hold their own, and then shrink quite rapidly again.

How much I was chagrined at first in not finding the curve a straight line, like that for the fatigue of a muscle, I will not stop to say.

Another point of great importance, viz. that the curve of Dr. Lombard, just referred to, was obtained from but a few minutes' work, whereas mine represents the fatigue of ten hours, I cannot discuss in full until my work upon the changes in the *living* ganglion cell under stimulation is completed. It will be sufficient for the present to remind the reader that the dots showing breaks in the curve at one hour, two and one-half hours, five hours, and ten hours are points taken in an entirely arbitrary manner. Had the observations been made every hour, or every half-hour, the curve might have passed through the same points and at the same time have been materially different. In other words, there is no reason to believe that, for example, just at the end of five hours fatigue began to be accelerated. This point may have occurred in reality at the sixth, seventh, eighth, or ninth hour, or at any time between. That is to say, the points of the curve may be averages of wide fluctuations occurring between them. Clearly, the only way to settle this point is to make the intervals of observation much closer together, or, as I hope to do more successfully than hitherto, to watch closely the living cell during a considerable period of stimulation.

By the continuous line in the figure is represented the process of recovery in the series of rest experiments (Table IX), in which five hours of severe work has caused a shrinkage of the nuclei of 48.8 per cent, recovery taking place as indicated by the second part of the curve. The curve of recovery, in this instance, is seen to rise quite rapidly at first, then more slowly, and again more rapidly to a point a little above the normal. This is the exact opposite of the view given by Landois and Stirling (39, p. 587): "When a nerve recovers, at first it does so slowly, then more rapidly, and afterward again more slowly." However, if depth of sleep may be taken to represent rapidity of recovery, then the curve given by Exner (12, p. 296) for depth of sleep, with the necessary reconstruction, corresponds not so badly with my own curve.

It is not strange or anomalous that the curves of fatigue and recovery should be in character alike, since both processes must be of a similar nature. That is, both are processes of the liv-

ing, active cell. The cell is perhaps as actively at work in phases of anabolic as in katabolic changes. Neither is it anomalous that these changes do not go on in the cell continuously and with equal steps. Perhaps no instance of living cells working thus continuously could be cited in all biology. Everything seems to be done in rhythm. And if the cells of a cleaving ovum pass through "resting stages" and "stages of activity"¹ (83, p. 292), and if the bodies of school children, as Bowditch² has shown, grow not continuously and equally, but now fast and again more slowly, there is every reason to suppose that nerve cells may follow the same rule. Thought, psychologists tell us, flows not continuously, but in waves. And general experience proves that the beat of the waves of thought is not equable and uniform, but variable in the extreme. Now they dash high, now they run in a gentle ripple, now there is the calm of stupidity or sleep. And may not thought be an index to the activity of nerve cells?

I have already stated that these curves are provisional. In fact, they have been introduced with the purpose of showing that they cannot be wholly relied upon, rather than of attaching permanent value to them. This is because an important factor in their shaping has been entirely ignored. This factor is, of course, the normal tendency toward activity or toward rest, toward anabolism or toward katabolism present in the cells while the stimulus is being applied.

From the first, we have been endeavoring to discover only such changes as occur in the normal functional activity of the nerve cell. That changes already described do relate to normal and not to pathological processes seems conclusively proved. If, then, these changes are normal, there should be no difficulty in demonstrating a similar rhythmic curve of rest and work in the normal daily activity of the animal. No more fundamental rhythm exists, either in physiology or psychology, than that of activity alternating with rest, sleep with waking. And this rhythm, from such work as Lombard (45, pp. 11 ff.) has done,

¹ Ref. W. K. BROOKS' "Alternations of Periods of Rest with Periods of Activity in the Segmenting Eggs of Vertebrates," *Studies Biol. Laborat.*, Johns Hopkins University, Vol. II., 1882.

² H. P. BOWDITCH, "The Growth of Children," Mass. State Board of Health, Twenty-second Annual Report, p. 509. Boston, 1891.

showing the influence of sleep upon volitional power, must be closely connected with, if not entirely dependent upon, events taking place in the central nervous system.

If any such rhythm exists in the cells of the spinal ganglia, it is evident that such curves as we have drawn may be profoundly influenced by it. A stimulation of five or ten hours is physiologically a trivial matter compared with a fundamental rhythm which has become through generations an established fact in the economy of an animal species; and if the changes in such a rhythm are similar to those which have been demonstrated by means of artificial stimulation, then clearly the effects in each case have been *resultants* between the influence of stimulation and the tendency of the animal's rhythm at the time. That is, if stimulation be applied while the animal's curve is falling most rapidly into sleep, we should not expect the same effect which would be obtained were the animal's curve of nervous activity on the rise.

A young dog stimulated *severely* for ten hours, from 5.30 P.M. until 3.30 A.M., showed scarcely a trace of fatigue. The purpose of the experiment was by no means to illustrate the point under discussion, but to obtain the greatest amount of change possible in the spinal ganglion cells. The result, at the time, struck consternation. Facts which it was hoped were of general application, fitting equally well the activity of nerve cells, wherever found, in the animal series from the highest to the lowest, must now be given most absurd limitations. This and this is true for the spinal ganglion cells of a frog or cat, but not for the same cells of a dog, and may or may not be true for man or any other animal. The shock of this unexpected result was paralyzing at first; and whether we are justified in saying that the tendency toward recovery, the tendency to sleep, in the cells of the spinal ganglia was strong enough to counterbalance an intense stimulus, which was sufficient to cause constant and vigorous contractions of the muscles supplied by the stimulated nerve, is doubtful, and plainly requires further experiment to decide. This seems to be, however, the simplest explanation of the phenomenon at present. And in saying this, the writer is fully aware that, more than two hundred years ago, Swammerdam studied reflex action in sleeping animals and men, and hence that the cells of the reflex arc must

be somewhat irritable in sleep¹ (43, p. 53 ; 58, p. 345 ; 39, p. 693).

A second dog, stimulated similarly for one hour and twenty-five minutes (10.05 to 11.30 A.M.), dying suddenly from the operation on the brain at the end of this time, showed most clearly the characteristic effects of fatigue. Hence, we are not compelled to make an exception in the case of dogs.

It is plain from the above considerations that a study of the normal rhythm of sleep and activity should be made for the animal employed in connection with further work of this kind. To this end I have kept under constant observation for a week a half-grown kitten similar to the ones used in my experiments. The sleep of such a kitten depends largely upon the amount of food given to it. If fed to repletion, it would sleep as much as eighteen hours a day, and, even when sparingly fed, slept twelve and one-half or thirteen hours. It seemed to be able to sleep equally well day or night. In short, the curve of nervous activity of a cat is most irregular.

It will be noted that if the cat possesses no marked daily rhythm of rest and activity, our provisional curves are more likely to be correct.

VII. EFFECTS OF NORMAL DAILY FATIGUE.

A crucial test as to the value of foregoing experiments for normal physiology is readily seen to lie in the question, Do changes in ganglion cells, like those observed during artificial stimulation, *actually occur* in the *normal activity of an animal*? If they do not, the experiments do not concern normal physiology of the nervous system. In spite of all proof to the contrary, they must be considered pathological. If they do occur, with the evidence already adduced, it will be but fair to consider them a part of the normal physiological activity of the nervous system.

¹ This experiment may have been further complicated by the fact that the pup, being of large breed and growing rapidly, lymph in great amount exuded from the wound and formed a pool in the axilla around the nerves and electrode. I did not notice this until quite late, when I thought that the contractions were becoming weak from fatigue. On wiping up the lymph more carefully, they became as strong as at first. In short, stimulation may not have been as "intense" as I had designed to have it.

A number of considerations combine to create a strong presumption in favor of the supposition that these changes will be found in normal activity. The processes in a gland have been found to be identical, whether produced by artificially stimulating the nerve going to it or by the normal stimulus of food. Electrical stimulation of a nerve causes contraction of muscle exactly similar to that produced by a normal nerve impulse. And here we have the normal impulse producing a stronger contraction than an electrical stimulus. If the same law holds good for centrally as for peripherally passing impulses, for sensory as for motor impulses, we should find a greater effect in sensory cells due to the normal stimuli of the animal's life than we are able to cause by stimulating an exposed nerve trunk. But, most of all, the phenomena of daily fatigue, so closely connected with the central nervous system, with the absolute necessity of not only rest but of long continued *sleep* for recovery of nervous power, is inexplicable on any ground which does not suppose profound changes within the central nervous system; and, knowing what we do as to the fatigue of nerve fibres, we may place these changes within the nerve cells themselves.

If normal daily fatigue is to be studied, first of all it is necessary to choose an animal in which a diurnal rhythm of rest and activity is highly developed. The cat we know is not such an animal, although the cat or other laboratory animals might be employed under the compulsion of some sort of exercising machine, and this may be done later. For the present, we wish distinctively to avoid all compulsion and to study only such activity as an animal normally and voluntarily puts forth in the ordinary round of its daily life.

In no animals is this daily rhythm more constant than in day birds and insects. In both of these classes, too, metabolic changes are known to be vigorous and rapid. The work done in a day by certain kinds of birds or insects is enormous, and could probably not be equalled, per body weight, by animals of any other group.

Method.

In a former communication (24, p. 331) the words occur, "It was found that the ganglion cells of two frogs that could not be distinguished externally might differ widely in staining and

general appearance." Probably the same statement holds good for individual birds and bees. Nevertheless, we are compelled to abandon this safe precaution of using only cells from different sides of the same animal. It would be clearly impossible to remove a spinal ganglion from one side of a bird or one half of a bee's brain in the morning and the corresponding parts at night, without seriously interfering with the animal's normal activity. Nothing of the sort was attempted. However, wherein the rigidity of the method is weakened by comparison of the cells of different animals, it is possible to strengthen it by making observations more numerous.

Aside from this the method of operation is essentially the same as that already described.¹ The birds, sparrows and swallows, were shot morning and evening at as nearly the desired time as possible, and the parts to be studied were excised on the spot. The pigeons were decapitated, no anæsthetic being used. A pair of spinal ganglia in each case were preserved in osmic acid, one per cent solution being used as formerly. The time was shortened to two hours' immersion on account of the small size of the ganglia. The other parts were preserved in saturated corrosive sublimate solution at 40° C. for four hours.

Both male and female birds were employed, but, with one exception, males were compared with males and females with females.

Results.

The following table gives the results of six experiments for the parts studied. Sections were taken perpendicular to the surface of the cerebellar and occipital cortex, longitudinal sections being made of the spinal ganglia.

The fact to strike one first upon examination of the specimens or the table is the great amount of change due to a day's fatigue. This is seen to exceed anything obtained by artificial stimulation in almost all cases. The highest per cent shrinkage of nuclei, 69.7 per cent, is found, strangely enough, in the occipital cortex of a female sparrow April 22, after a long

¹ One thing, however, has escaped my attention, viz. the hardening, in osmic acid, of the specimens to be compared was not done at constant temperature. A slight difference, hence, between morning and night temperatures may have had some influence upon the results. That this difference has not complicated matters seriously is shown from the fact that other portions of the same animal hardened in corrosive sublimate at 40° C., and hence, not amenable to temperature variations, give results equally good.

TABLE X.

SERIES OF EXPERIMENTS TO SHOW EFFECTS OF A DAY'S NORMAL ACTIVITY IN THE CELLS OF DIFFERENT PARTS OF THE NERVOUS SYSTEM.

(Corresponding parts in each animal treated in the same manner and compared with each other.)

EXPERIMENT.	TIME.	OCCIPITAL CORTEX.		PURKINJE CELLS, CEREBELLUM.		SPINAL GANGLIA.	
		Mean diam. of nuclei.	Shrink- age.	Mean diam. of nuclei.	Shrink- age.	Mean diam. of nuclei.	Shrink- age.
I.							
(Dec. —, '91.)							
<i>English Sparrow.</i>							
1, male.....	7.00 A.M.					12.04 μ	
2, "	5.30 P.M.					9.99 μ	54.3 %
III.							
(Feb. 17, '91.)							
"Rainy day."							
<i>English Sparrow.</i>							
3, female.....	7.00 A.M.	8.09 μ		8.06 μ		No difference observ- able, hence not measured.	
4, male.....	4.30 P.M.	6.72 μ	43 %	7.75 μ	8%		
IV.							
(Apr. 22, '91.)							
<i>English Sparrow.</i>							
5, female.....	6.30 A.M.	6.69 μ		8.31 μ		10.69 μ	
6, "	6.30 P.M.	4.43 μ	69.7 %	6.85 μ	43%	7.44 μ	64 %
II.							
(Dec. —, '91.)							
<i>Pigeon.</i>							
1, male.....	8.30 A.M.					15.34 μ	
2, "	5.30 P.M.					12.82 μ	49.5 %
V.							
(Apr. 28, '91.)							
<i>Pigeon.</i>							
3, female.....	5.30 A.M.	10.59 μ		12.74 μ		13.88 μ	
4, "	7.30 P.M.	9.19 μ	36 %	10.32 μ	51.7%	11.62 μ	33.3 %
VI.							
<i>Swallow</i>							
<i>(H. horreorum).</i>							
(June 10, '91.)							
1, male.....	5.00 A.M.	8.85 μ		9.12 μ		12.00 μ	
2, "	8.00 P.M.	6.84 μ	55.5 %	6.32 μ	64.5 %	9.82 μ	45.2 %

hard day of nest-building. An egg was found in the lower portion of the oviduct. The next highest percentage, 64 per cent and 64.5 per cent, expresses the amount of fatigue in the spinal ganglion cells of the same bird and in the cells of Purkinje, a male swallow, June 10. Barnyard pigeons, fed a little grain twice a day, show considerably less fatigue than the wild birds.

As far as my work would permit, some account of the activity of the birds was kept during the day of an experiment; and a day suited to the purpose of the experiment was chosen.

Experiment I was made early in December, toward the end of a cold blustering snowstorm. Sparrows keep under pretty close cover while such a storm continues, and at its close may be seen out in force and actively in search of food. Advantage was taken of a case of this kind; and the difference between the cells of the spinal ganglia, the only part taken, morning (Fig. 6) and evening (Fig. 7), is readily seen by comparison. Although not showing the highest shrinkage per cent, the cells of sparrow 2 (Fig. 7) do present a somewhat more striking state of dilapidation than those of sparrow 6, and hence were chosen for the plate. I suspect also that an individual complication is present here, in the way of incipient starvation, as the crop of this sparrow was empty, and there was little food in the gizzard, and this at night when both are usually well filled. The protoplasm is seen to be extremely vacuolated and the nuclei much shrunk. The peculiar clear spaces which form such a marked feature in the cells of sparrow 1 (Fig. 6) are somewhat aside from the line of our thought at present, and will be discussed on a later page.

Experiment II was made about the same time, and is simply confirmatory of Experiment I. Shrinkage of the nuclei in the pigeon is nearly as marked as in the sparrow. Vacuolation of protoplasm is not so striking, although present.

Experiment III deserves special remark. It was made with the single purpose of confirming Experiments I and II. But on the morning of February 17, shortly after sparrow 3 had been shot, it began to rain, and continued nearly the whole day, a steady, warm, foggy spring rain. In the dense cover of the pine trees over my window the sparrows spent the day scolding and chattering at a great rate. None were observed flying about. At first I decided to abandon the experiment, thinking

that I would find little evidence of fatigue on such a day. On second thought, however, I concluded to make a "rainy day" experiment of it and see what might be the result. I little expected the sharp and somewhat amusing result expressed in the table. Not an observable sign of fatigue was to be seen in the spinal ganglia; while traces of fatigue were slight in the cells of Purkinje. Perfectly clear, however, were the marks of fatigue in the nuclei of the occipital cortex, as though, while confined by the rain, the little birds had kept up a deal of thinking. The experiment is further complicated by the fact that upon the night of February 14, in accordance with the time-honored custom of St. Valentine's day, the boys had "shelled" the windows of Worcester with peas. The subsequent thaw had left them soft and swollen upon the surface of the snow; and as a result the crops and gizzards of the sparrows on February 17 were filled with peas both morning and night. Indeed, it would require but trifling effort on such a day of plenty for a sparrow to lay in a supply of food sufficient for several stormy days.

In order to have represented in the plates as many experiments upon as many of the different animals as possible, Figs. 8 and 9 were taken from the occipital cortex of Figs. 3 and 4. These figures show fairly well the difference between the morning and evening cells of the other birds.

It will be specially noted (Figs. 8 and 9, and 12 and 13) that whereas, in spinal ganglion cells with capsules, loss of substance in the protoplasm is shown by vacuolation with little shrinkage of cell, in the cerebrum and cerebellum the cells shrink bodily. This is expressed in part at least by enlarged pericellular lymph-spaces.

Experiment IV was purposely made upon a warm, bright day, April 22, when the sparrows were most busily at work nest-building, with purpose also upon female sparrows.

"Für den Spatz ist das Plaisir,
Für die Spätzin sind die Pflichten!"

Effects of the day's work are seen from the table to be quite evenly distributed over the parts of the nervous system examined. This is true for all cases except for No. III, the rainy day experiment

Experiment V was made for purposes of confirmation simply, and calls for no special mention.

Perhaps the most active bird that we have is the swallow. Its food consists of insects taken entirely on the wing. Quick, vigorous, purposeful, careful in all its actions, it must require an enormous amount of nervous energy to co-ordinate its countless movements for a long summer's day. All day long, whenever I chance to look up from my work, I see this bird flitting and sailing and circling, fluttering up and swooping down. There is nothing lazy or stupid about the swallows. When their work is done, they play games and fly races; and with all the energy required for flying, they have enough left to do no end of talking; for their cheerful "zwitschern" is continually in my ears while I write. At one hundred miles an hour, for ten hours, — and I have observed them as early as five o'clock in the morning, and as late as eight at night, — a swallow might cover a distance of one thousand miles in a single day, and day after day. If a bullet of the same weight were to traverse the same distance at the same speed, an enormous explosion of energy would be required, and the living arrow can require no less.

Accordingly, for Experiment VI, swallows were employed.¹ A day was chosen, when weather predictions were favorable, at a time (June 10) when swallows are busiest feeding their young. I reached Coes' Pond in the morning, before a swallow was in sight. At just five o'clock, a large male swallow flitted from the eaves of an ice-house, and, alighting on a telephone wire, began preening his feathers for his morning flight. Within five minutes, his brain and spinal ganglia were in their proper hardening fluids, osmic acid and mercuric chloride.

Again, at a little before seven, I took my stand by the same pond. Swallows were circling thick. I waited until a few minutes before eight, when all but two, both males, had gone home for the night. One of those flitted too close to my gun, and came down with a broken wing; and by eight o'clock his brain and ganglia were treated like those of his brother of the morning. I could not, however, help making the note, as

¹ The writer takes pleasure in acknowledging the courtesy of Messrs. E. A. Brackett and Edward H. Lathrop, Commissioners of Fish and Game for the State of Massachusetts, in granting the official permit under which these birds were killed.

I watched them flying at evening, "They don't seem tired one bit."

From results of experiments upon birds, with the great amount of matter lost from the nervous system during a day's work, I feel confident in chancing the prediction that a small, active bird, an English sparrow, for example, could not be kept awake and fluttering a single night without fatal results. I had hoped, instead of the prediction, to have been able to report an experiment of this sort; but time and the opportunity have not been conjoined thus far.

In addition to signs of fatigue present everywhere in the parts examined, the brains of these swallows held in waiting an agreeable surprise. By reference to the table (X, Exp. VI), it will be noticed that the cerebellum shows the highest per cent of loss, nearly ten per cent more than the occipital cortex. The same thing is true of the pigeon, but not of the sparrows. Extreme cases naturally make a much stronger impression than mean cases of nearly the same magnitude; and such an extreme case has been shown in Fig. 12 (Pl. VIII), taken from the cerebellum of the night swallow. It is to be compared with Fig. 13, drawn from the morning bird. Cells could easily have been selected for measurement which would have shown a much greater percentage of loss; but, this not being allowable, the figures in the table give presumably a fair average, while Figs. 12 and 13 present the extremes. From the figures, too, the nuclei of Deiter's cells are seen to have shrunk, as well as those of Purkinje. To the cerebellum is generally ascribed the work of muscular co-ordination, and where could be sought an instance of more delicate manipulation of muscles than must be required to drive the wing of a swallow as it flits and whirls and balances and wheels and darts, the whole day long? In the pigeon and sparrows, although the nuclei of the Purkinje cells show great shrinkage, these extreme cases are not met with. These birds use their legs as well as wings.

To discuss a result of this kind, however, carries us far ahead of our present purpose and knowledge. It is exactly what might have been expected, had the idea occurred; yet, now that it stands before us, we are afraid to believe it; and will promise not to, until further experiment is made. But the time may come when we shall be able to study some phases of local-

ization in the brain by means of changes in the cells due to fatigue.

The pigeons were not introduced solely to add variety to the list of animals used; but with a distinct purpose of another kind. Arrangements had been made with a pigeon fancier¹ of Worcester, to furnish a number of trained homing pigeons. These birds, if taken away from their loft and liberated, are said to fly without alighting during the first day, or until the loft is regained. Records have been scored of over five hundred miles, air-line distance, on the day of liberation (77, p. 366), the birds coming to loft, I am told, too fatigued to hold up their wings from the floor. It was intended to make at least one experiment with them to show extreme fatigue, fatigue from which, I am informed, pigeons require not only a night's sleep, but several days' time, to fully recover. The birds were lost in course of training, and I was obliged to leave Worcester before others could be obtained.² I had intended using the common pigeons as normals, to show the effect of a moderate day's work, for the homing pigeons, which, it was hoped, would demonstrate, by comparison, extreme fatigue.

Failing of the homing pigeons was possibly for the present a piece of good fortune, for I bethought myself of another animal, the proverbially "busy bee." From these, at any rate, I have obtained most striking results. They may be seen at a glance by comparing Fig. 10 (evening) with Fig. 11 (morning).

On the morning of June 10, after securing my swallow, I stationed myself by a patch of raspberry bushes in full bloom and within a stone's throw of a small apiary, and watched for the bees to come. At six o'clock sharp they came. The first

¹ The writer refers to Mr. Frank Keith, to whom he is under great obligations for kind assistance and valuable information regarding the use of homing pigeons.

² Homing pigeons are expensive, when well bred, costing, minimum price, six dollars per pair, for young birds. Their training may cost an indefinite amount more. I have, however, to thank Dr. S. Weir Mitchell for a fine loft of about thirty blooded homing pigeons; a number of which are being trained at this writing for longest possible flights in order to furnish material for the above-mentioned experiments. It is, however, a much longer undertaking than I anticipated. The birds attain full maturity in not less than four years. Young pigeons lack the mental development, the grit, and perseverance, to put forth the great amount of effort desired. But the experiments will be reported in due time.

six bees I could catch were quickly decapitated, the brains removed, and three were dropped into one-half per cent osmic acid, and three into saturated mercuric chloride solution.

While watching the swallows the same evening, I caught six bees at about seven o'clock. These were laid aside in a net, and with a second net I caught six more. I then released the first six and repeated the operation; until, at about half-past seven, when no more bees could be found on the flowers, I retained the six bees last captured. Before taking their brains, I watched them for the space of perhaps ten minutes. Five sat perfectly still in the net; one buzzed angrily and without cessation the whole time, in fact until his head came under the scissors. This one was named "lively bee," and his brain was kept separate from the rest. The brains were treated, of course, like those of the morning lot.

The preparation continued until eighty per cent alcohol was reached, when the morning brains were allowed to remain enough longer to catch up; and then all were arranged in pairs upon slips of cardboard, as described on page 115. With the exception of No. 12 ("lively bee"), they were paired indiscriminately, osmic acid brains, morning, with osmic acid brains, evening; the mercuric chloride specimens in the same way; and for convenience they were numbered, the odd numbers representing morning, the even numbers evening bees.

The attempt was made to measure the nuclei after the manner of foregoing experiments; and although one may see from Figs. 10 and 11 how far from satisfactory such a method might be, still the results will be given in tabulated form.

TABLE XI.
HONEY-BEE EXPERIMENTS.

		ANTENNAL LOBE.		
	Number of bee.	Mean diameter of nuclei.	Per cent of shrinkage.	
Osmic Acid.	1.....	4.53 μ		
	2.....	<u>5.25</u> "	64 %	
	Diff.....	1.28 "		
	3.....	4.09 "		
	4.....	<u>2.94</u> "	73 %	
	Diff.....	1.15 "		
	5.....	4.65 "		
	6.....	<u>3.25</u> "	73 %	
	Diff.....	1.40 "		
	7.....	4.60 "		
	8.....	<u>3.90</u> "	34 %	
	Diff.....	.70 "		
Mercuric chloride.	9.....	4.56 "		
	10.....	<u>3.96</u> "	33 %	
	Diff.....	.60 "		
	11.....	4.46 "		
	12 ("Lively bee").....	<u>4.35</u> "	8 %	
	Diff.....	.11 "		
			Minimal (barring No. 12).	
	3.....	4.09 μ		
	10.....	<u>3.96</u> "	9 %	
	Diff.....	.13 "		
			Maximal.	
	5.....	4.65 μ		
4.....	<u>2.94</u> "	75 %		
Diff.....	1.71 "			

Arranged, as they were, at random, we have the right to pair any morning bee with any evening bee. This gives us as a minimal shrinkage nine per cent, as a maximal seventy-five per cent. Although I do not attach exact values to these figures, they express a truth easily observed in the specimens; viz. the wide difference between them. The fact is brought out by comparing morning bees with morning bees, and evening bees with evening bees. Here we observe that the morning diameters, 4.09, 4.53, 4.46, 4.56, 4.60, 4.65, are somewhat more uniform than the evening diameters, 2.94, 3.25, 3.25, 3.90, 3.96 (4.35); the greatest difference between morning diameters being .56 μ ,

between the evening diameters 1.02μ , barring No. 12 (with No. 12 1.41μ); while the greatest difference between morning and evening diameters is 1.71μ .

Did I feel that the above figures were more trustworthy, I would go into their manipulation more in detail. Enough has been given to make plain the following points. First, the nerve cells of a number of bees' brains are in a more uniform condition in the morning than in the evening. Second, they differ in appearance, or condition, from one another somewhat in the morning and a great deal in the evening. Working bees from the same hive would strike one as being as much alike as it would be possible to conceive of a number of animals. Whence then are these differences?

No individual difference of size was noticed. All honey-bees which are out gathering honey from the flowers must have an abundance of food on hand; and the food of bees in a given place and time must be the same. Hence no differences in nutrition would be likely to occur.

If six bees were exactly alike in the morning, their brain cells, of course, should appear alike, if examined by the same method. If all the six should fly exactly the same distance in the same time, *i.e.* do exactly the same amount of work, we should expect to find their brains in the same condition again at night.

There are two important variables present which unfortunately we know little about. *If* the bees are alike; *if* the work is alike. The work may vary; the bees may vary within indefinite limits.

With reference to the amount of work done by a bee, we know almost nothing. Lubbock (46, p. 276) and the Peckhams (65) have counted the number of trips a bee or wasp may make in a day, and this number varies; but who has ever followed a bee in one of its flights? Whether a load of honey be found near or far away must cause the flights to vary. Still, it is evident, these two variables, length and number of flights, may be so combined as to produce a constant amount of work.

When a boy in college, the writer owned some bees. Every morning, in the busy season, a few bees could be found dragged out of the hive dead. Every evening might be seen in the grass near a hive, bees with the frayed wings and abraded hairs betokening old age, heavily laden, but too tired to lift them-

selves in the air for the short space necessary to regain the hive. With food and parentage and every element of living so exactly alike, observations like the above have led me to think that the only difference between the bees in a hive, a difference which might bring about a complication of results like that occurring in Table XI, must be a difference of *age*. This would naturally lead to a difference of work.

Figs. 11 and 10 (Pl. VIII) are drawn respectively from bees 3 and 4. Although paired together by accident, they serve to illustrate my point better than any of the others. In No. 3, Fig. 11, we notice that in cells of about the same size the size of the nuclei varies considerably, and a good many appear shrunken and somewhat angular in outline. In all the other morning bees they are more uniform. Is it not possible that this is the case of an old bee, in which the balance between repair and waste has turned toward the side of waste? The night's rest is no longer sufficient for complete recovery from loss due to the day's work. Bee No. 4 (Fig. 10) is the extreme case in the series. In no other one are the nuclei quite so shrunken and the cell protoplasm so extremely vacuolated. I cannot do less than make the remark regarding this bee, that possibly it might have fallen by the hive to die that night.

Bee No. 12 is an evening bee that shows, so far as brain cells or actions go, no signs of fatigue. If I were given a section of any of the other bees' brains and asked: "Morning or night?" I could tell which. With this one I should say, "Morning." In strictest logic, therefore, I am obliged to say, that in five cases out of six the cells of bees' brains show, at night, effects of the day's fatigue. In one case in six this does not appear. My own supposition, however, is that No. 12 is a young bee, out for a stroll in the cool of the evening.¹

The antennal lobes were chosen for special study, because the cells were uniform in size, shape, and grouping, and were easily located so that certainty of comparing similar parts was attained. Other regions presented similar appearances, but

¹ The writer's regret for neglecting to observe "age signs" in the above bees can better be imagined than expressed. However, experiments are under way to remedy this defect.

were less regular and well defined. The lobes were located by the aid of Riley's (71) description of the locust's brain.

Besides those tabulated, several preliminary experiments were made, two upon bumble-bees and two upon honey-bees. As these all show evening fatigue, the ratio of fatigue cases is much increased.

This closes the list of diurnal fatigue experiments. The writer regrets the absence of a mammal from the series. One experiment upon a dog was attempted, but terminated unfortunately.¹ I hope, however, in the near future to be able to make some experiments upon mammals which shall supply this deficiency. At present I have the following observations to append.

The "Worcester Fur Company" is an organization of gentlemen upon the principle that foxes should be chased at least one day in the year. At their meet the Company placed two of the carcasses at Dr. Donaldson's disposal. The brains were used for comparative anatomy specimens. I obtained spinal ganglia of each, which, compared with those of a dog of about the same size, show nearly as much difference as is seen between Figs. 1 and 2. No data were obtained as to how long the foxes had been chased. The method of hunting in that section being to shoot the fox at sight, no estimate of this quantity can be made. They may have been shot as they jumped from cover or after the hounds had chased them for several hours. Signs of great fatigue, compared with what has been found in birds and bees, are certainly not present.

Without exception the motor cells in the ventral horns of human spinal cords that have come under my observation present considerably shrunken nuclei. In the spinal cord of a hydrophobia patient,² however, this phenomenon is presented in an extreme degree. Characteristic ecchymoses in the gray matter were numerous (17, Vol. II, p. 847). According to Gowers, changes in ganglion cells in hydrophobia are trivial. Popow (66) in a single case notes little of interest to us

¹ After working the dog from five o'clock in the morning until three in the afternoon, racing him through woods and swimming him in ice-water, which he did willingly, the dog bolted and was not seen again for three months.

² For the above material I am indebted to the courtesy of Dr. R. H. Chittenden of New Haven, Conn.

except pigmentary degeneration ; and this may occur in almost any specimen. No special amount of pigment was remarked in the case in hand. Measuring a set (in this case 20) of nuclei in a so-called normal cord for comparison gave the following result : —

Nuclei of normal cord ; mean diameter	. . .	4.30 μ
Nuclei of hydrophobia cord ; mean diameter	. . .	3.12 μ
Volume per cent smaller	. . .	59 %

I throw out the above merely as a straw which may serve to show the direction of the current. We may have further use for the material at some future time.

I cannot close without mentioning by way of preliminary communication the peculiar appearances found quite constantly in osmic acid preparations of the ganglion cells of birds. They are represented in Fig. 6, drawn throughout by the aid of a camera lucida. A few are seen in Fig. 7 ; viz. the vacuoles with sharp outlines and definite shape. The majority of the vacuoles in Fig. 7 are easily seen to differ in these respects from those in Fig. 6. In corrosive sublimate preparations they are seen to be present, but are masked by granules.

When first noticed, their definite form was thought to indicate bodies of a crystalline nature in the protoplasm of the cells. They were accordingly tested with polarized light, but were found to be inert.

Altmann (2) states that, whereas fats on treatment with osmic acid become insoluble in alcohol, certain fatty acids remain soluble. Therefore tissues hardened in osmic acid, if they contain droplets or crystals of fatty acid after dehydration in alcohol, present *vacuoles* holding the shape of the fatty acid particles. It was thought that this might account for the lack of any action upon polarized light. Accordingly a fresh morning sparrow was taken and the ganglion cells crushed out quickly and examined under polarized light. The result was doubtful. If any crystalline bodies were present, they vanished almost instantaneously. In the liver, among fat droplets, which shone brightly on the dark field, were a few shining particles shaped like those in the ganglion cells, but these were quite permanent. The same forms are found in the osmic acid liver. In the oil gland, freshly crushed out, among sheaves of fatty

acid crystals, particles of the above form were quite numerous, but these also had no tendency to vanish.

Grandis (16) has obtained staining of intranuclear crystals by long immersion in osmic acid. This was also tried, teasing out the ganglion cells in osmic acid, but with uncertain or negative results. Miss Leonard (41, p. 39) also calls attention to crystals or crystal-like bodies in the liver cells of frogs.

Appearances of this form have a somewhat wide distribution in avian tissues, so far as examined. I have found them in the spinal and sympathetic ganglia of all birds studied, in the livers of several, all which were examined for them, in the uropygial gland of two (only ones examined), and in the secreting cells of the oviducts of two fowls. They are most numerous in the ganglion cells and oil gland, and occur somewhat sparsely in the other locations. Absolute identity in these different cases is, of course, not established, farther than such identity is indicated by similarity between the forms observed.

In general, as indicated in the figures (6 and 7, Pl. VIII), these figures are numerous in morning cells and fewer in those of the evening, their place being represented by more or less irregularly shaped vacuoles. It is as impossible to stain them as it is to stain the vacuoles of the evening cells. In fact, as they exist in the sections, I suppose they must be considered vacuoles; their uniform and definite shape, however, indicates that they are produced by solution of some formed substance in the cells. That they cannot be artifacts is proved by their form and arrangement in the cells, by their difference in size in different cells, by their greater numbers in morning material, and by their entire absence from frog and mammalian tissues, treated by the same methods.

I will not attempt to describe these appearances more in detail as to shape, size, and origin until further experiments are made.

CONCLUSIONS.

Metabolic changes in nerve cells are certainly as easy to demonstrate, microscopically, as similar processes in gland cells. They may be demonstrated equally well, and are the same in character, either by artificial or natural methods.

The principal changes thus far observed are : *for spinal gan-*

glion cells of frog, cat, dog, under electrical stimulation; for spinal ganglion and brain cells of English sparrow, pigeon, swallow, and for brain cells of honey-bee, under normal fatigue:—

A. For nucleus: 1. Marked decrease in size. 2. Change from smooth and rounded to a jagged, irregular outline. 3. Loss of open reticulate appearance with darker stain.

B. For cell-protoplasm: 1. Slight shrinkage in size, with vacuolation for spinal ganglia; considerable shrinkage, with enlargement of pericellular lymph space for cells of cerebrum and cerebellum. 2. Lessened power to stain or to reduce osmic acid.

C. For cell capsule, when present: Decrease in size of nuclei.

D. Individual nerve cells, after electrical stimulation, recover, if allowed to rest for a sufficient time. The process of recovery is slow, from five hours' stimulation, being scarcely complete after twenty-four hours' rest.

E. Provisional curves have been constructed from direct observations of the nerve cell to represent the processes of fatigue and recovery. These curves indicate that the nerve cell tires or rests rapidly at first, then slowly, then more rapidly again. That is, the curve of nerve-cell rest or fatigue is not a straight line.

I part with this manuscript with the feeling that I have not done justice either to my material or to the subject. Interruption has been unavoidable, and stress of other work great. It is, at best, but a small beginning in a field the bounds of which have opened out much faster than I have been able to advance. With greater opportunity and facilities for work which Clark University will afford, it is to be hoped that something may be accomplished during the coming year.

In order to properly define results already obtained, it will be necessary to know two things. First, exactly what changes take place in nerve cells under variations of food and water supply. Second, what changes, if any, take place in nerve cells from birth to death from old age, from "rejuvenation" to "senescence."¹

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Aug. 27, 1892.

¹ An abstract of the above paper with demonstration of specimens was given before the American Physiological Society at the Congress of American Physicians and Surgeons, Washington, D.C., September 22, 1891.

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EXPLANATION OF PLATE VII.

ELECTRICAL STIMULATION. — CATS.

FIG. 1. *Normal*. Cat 17. Left spinal ganglion of 1st thoracic pair. Osmic acid.

FIG. 2. *Stimulated 5 hrs.* Cat 17. Mate ganglion to Fig. 1. Osmic acid.

By comparing Fig. 2 with Fig. 1 is seen the effect of severe work (15 seconds' stimulation to 45 seconds' rest) for 5 hours, the nuclei becoming darker, shrunken and irregular in outline, protoplasm somewhat vacuolated.

FIG. 3. *Normal*. Cat 17. Three cells from left ganglion of 8th thoracic pair. Corrosive sublimate, 40°; 4 hrs. Gaule's quadruple stain.

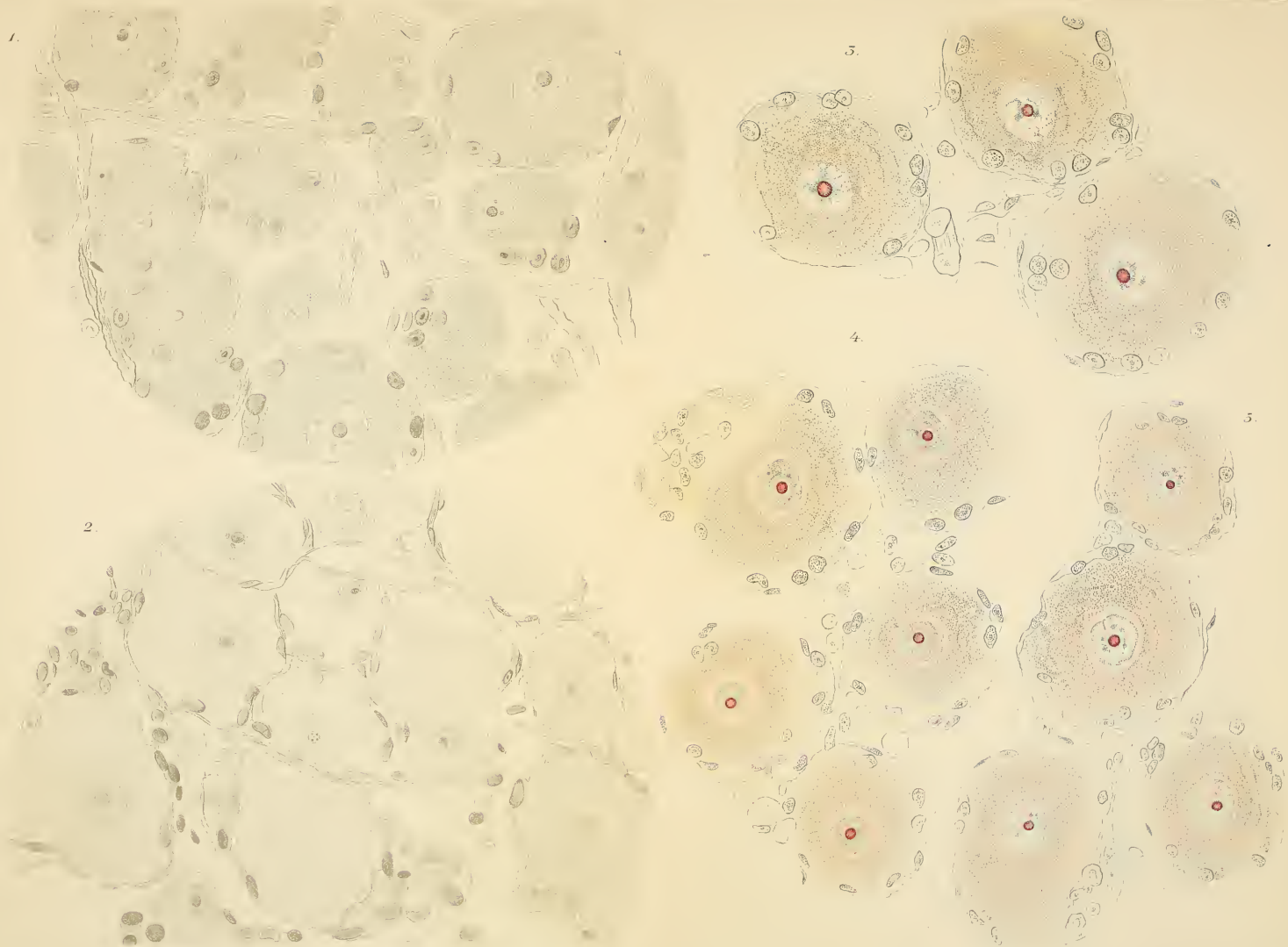
FIG. 4. *Stimulated 5 hrs.* Cat 17. Five cells from mate ganglion to Fig. 3. Like treatment.

Compare Figs. 3 and 4 for effect of stimulation upon size and character of nucleus.

FIG. 5. *Rested 6½ hrs.* Cat 16. Four cells from right 8th cervical ganglion, stimulated 5 hrs, rested 6½ hrs. The normal of Fig. 5 is like Fig. 3. Preparation same as for 3 and 4.

Compare Figs. 5 and 4 for influence of rest.

The above, Figs. 1-5, were drawn under magnification of Zeiss apochromatic, oc. 4, obj. 2 mm., oil immersion ($\times 500$ diameters). Outlines drawn by aid of Zeiss camera lucida, after Abbe (with longer arm). Cells of each figure contiguous, as shown by connective tissue, etc., uniting them.



EXPLANATION OF PLATE VIII.

NORMAL DAILY FATIGUE. — BIRDS AND BEES.

FIG. 6. *Morning*. Portion of field from 3d brachial ganglion of English sparrow, killed December —, '91, at 7 A.M. Osmic acid, 1 %, 2 hrs.

FIG. 7. *Evening*. Field from corresponding ganglion of English sparrow, killed same day (as Fig. 6), at 7.30 P.M. Like preparation with Fig. 6.

Figs. 6 and 7 demonstrate extreme daily fatigue with probably some lack of food. The queer-shaped clear spaces in Fig. 6 are seen to be replaced to a great degree in Fig. 7 by faintly outlined, irregular vacuoles. Nuclei (Fig. 7) appear shrunken, as in cases of electrical stimulation.

FIG. 8. *Morning*. { Occipital cortex of pigeons. April 28, '91; killed at 5.30
A.M. and 7.30 P.M. Corrosive sublimate, 40° C. 4 hrs.
FIG. 9. *Evening*. { Sections 3 μ thick. Gaule's stain, on the slide.

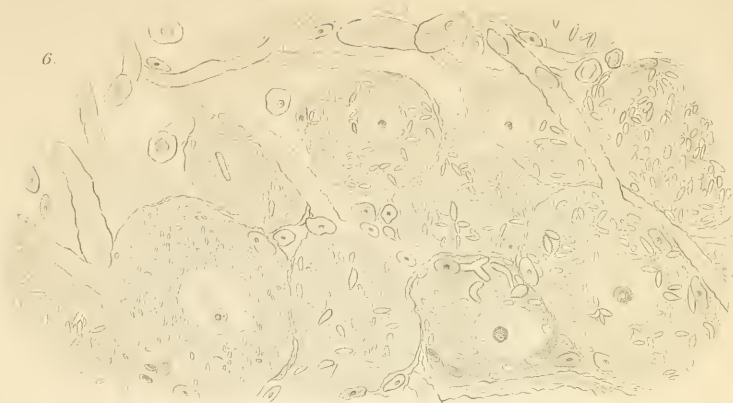
FIGS. 6, 7, 8, and 9, camera lucida drawings, magnification Zeiss, oc. 6, obj. 2 mm., oil immersion ($= \times 750$ diameters), apochromatic system.

FIG. 11. *Morning*. { Median subdivision antenary lobe of brain of honey bee.
Taken June 10, 6 A.M. and 7.30 P.M.
FIG. 10. *Evening*. { Osmic acid $\frac{1}{2}$ %, 2 hrs. Sections 3 μ thick, stained in slide
with Gaule's quadruple stain.
Camera lucida drawings, under Zeiss, oc. 8, obj. 2 mm., oil
immersion ($= \times 1000$ diameters).

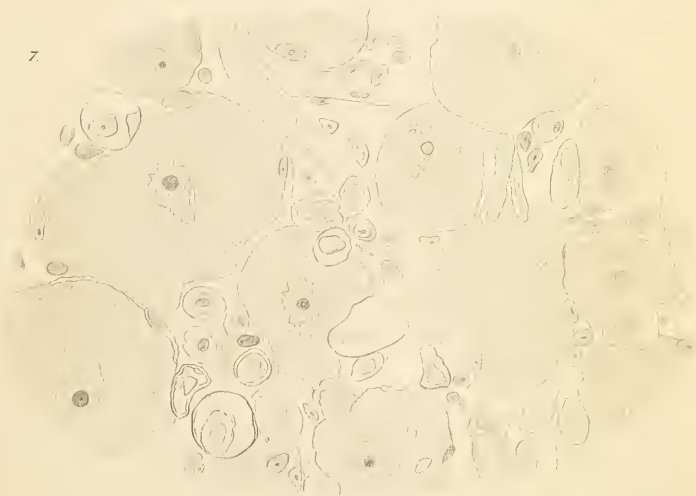
FIG. 13. *Morning*. { Cerebellum of swallows killed 5 A.M. and 8 P.M.
Corrosive sublimate, with Gaule's stain.
FIG. 12. *Evening*. { Camera lucida drawing, with Leitz, $\frac{1}{2}$ oil immersion, obj.
oc. 3 ($= \times 965$ diameters).



6.



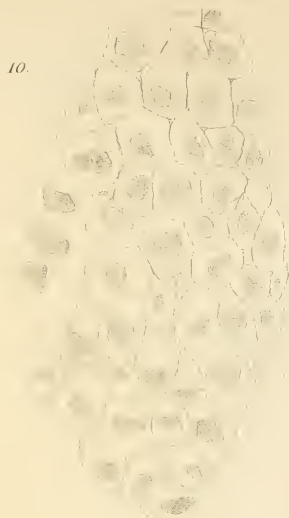
7.



12.

13.

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9.

11.



8.



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